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BLOOD

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This number of the Bulletin is a review of some recent work on blood and its disorders. The authors of the first paper, Dr. G. L. Taylor and Dr. R. R. Race, were until 1939 *working on the serological aspect of human inheritance with Professor R. A. Fisher at the Galton Laboratory, University College, London. Brief reference to this work was made in BMB* **346**. *On the outbreak of war, in anticipation of heavy demands for test-sera from military and civilian transfusion services, the Galton Laboratory unit was taken over by the Medical Research Council to form a Blood Grouping Serum Unit. In this period Dr. Taylor and Dr. Race have both published a number of papers on blood-grouping and other serological subjects, most of which are reviewed in this number.*

Dr. W. T. J. Morgan is a member of the staff of the Lister Institute of Preventive Medicine, and Reader in biochemistry in the University of London. He has been an Honorary Secretary of the Biochemical Society since 1940 *and was, from* 1929 *until* 1937, *First Assistant and Biochemist to the Institute's department for the preparation and study of therapeutic sera. His published work during the last* 10 *years has included many papers on immunochemistry. His chief interest has been in problems associated with the isolation of bacterial and tissue antigens in homogenous condition and yet retaining in full their "native" character. Dr. Morgan recently succeeded in combining non-antigenic polysaccharides which possess serological properties of biological interest, such as bacterial haptens, plant gums and specific blood-group substances, with certain bacterial proteins, thus forming potent antigenic complexes which induce the formation of immune-body specific for the polysaccharide component of the artificial antigen. The formation of artificial antigenic complexes under what are almost physiological conditions, opens up a new line of approach to the whole problem of the nature, formation and occurrence of antigens in micro-organisms and animal tissues.*

Dr. C. J. C. Britton is a graduate of the University of Otago, New Zealand. He first came to England in 1932 *for two years as a Research Scholar to the Bland-Sutton Institute of Pathology, Middlesex Hospital, London. Then, following three years as Assistant Pathologist to Christchurch Hospital, New Zealand, he was appointed Assistant Pathologist in the Bland-Sutton Institute and is now also Officer in Charge of the Emergency Public Health Laboratory, Sector* 5 *(one of the laboratories of the Emergency Public Health Laboratory Service, which was described in BMB* **64**). *Most of his published papers deal with hæmatological subjects. He is also co-author with Dr. L. E. H. Whitby of "Disorders of the Blood" (4th edition,* 1944), *and contributes the section on blood disease in Savill's "System of clinical medicine" (12th edition,* 1944).

Dr. Harold Scarborough is Temporary Assistant Physician in the Edinburgh Royal Infirmary. Formerly the holder of a Beit Memorial Fellowship for Medical Research, Dr. Scarborough has published work on a variety of nutritional subjects. He has made a detailed study of many problems involving capillary resistance, particularly in relation to the bleeding diseases. Since the war, Dr. Scarborough has been Principal Medical Officer and Deputy Director of the South-East Scotland Blood Transfusion Service. An account of a series of his papers on the properties of stored blood has already been given in BMB **94**.

Dr. E. P. Sharpey-Schafer is First Assistant in the Department of Medicine of the British Postgraduate Medical School, London. Before the war he was particularly interested in endocrinology, and had published a number of papers on this subject. Since the war his interest has been diverted to problems in connection with the dynamics of the circulation.

SPECIAL CONTRIBUTIONS

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HUMAN BLOOD GROUPS

G. L. TAYLOR, M.D., Ph.D., F.R.C.P.
and
R. R. RACE, M.R.C.S., L.R.C.P.

Human erythrocytes contain many different antigens (agglutinogens), usually demonstrated by agglutination on addition of serum containing corresponding antibodies (agglutinins). Besides antigens common to man as a species, there are others, present in some people and not in others, which enable us to divide mankind into several different systems of blood groups. The number of detectable antigens will no doubt be increased.

For many years it had been known that the serum of one species agglutinates the cells of another species of animal (heteroagglutination) when Landsteiner (1900), by finding that the sera of some human beings agglutinates the cells of others, showed there were differences in the blood of members of the same species. The agglutination of the cells of one member by the serum of another member of the same species is isoagglutination.

ABO Groups

According to whether the cells contain one (A), the other (B), both (AB) or neither (O) of two antigens, A and B, Landsteiner and his colleagues classified mankind into four groups. Of all human erythrocyte antigens, A and B are the only ones for which the corresponding agglutinins occur naturally in human serum, and because antigen and corre-

sponding antibody may come together *in vivo*, the ABO groups are of great importance in blood transfusion. Landsteiner pointed out that a person's serum cannot contain the antibody (agglutinin) for an antigen (agglutinogen) present in his erythrocytes, but with very rare exceptions anti-A or anti-B agglutinins (or both) are found in the serum when the erythrocytes do not contain the corresponding antigen. The position is shown in the following Table:

TABLE I

Antigens in erythrocytes	O	A	B	AB
Antibodies in serum	Anti-A + Anti-B (alpha + beta)	Anti-B (beta)	Anti-A (alpha)	None
Jansky classification	I	II	III	IV
Moss classification	IV	II	III	I

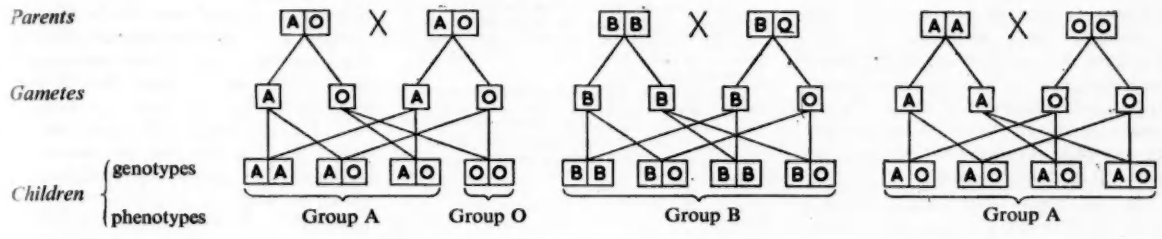
Instead of the alphabetical A, B, AB and O (O indicating absence of A and B) classification, based on the antigenic content of the cells, many workers, particularly in the past, have used a numerical classification. Unfortunately there are two different ways of numbering the groups, Jansky (1907) and Moss (1910), and confusion and accidents have resulted. Numbers should not be used for, apart from preventing confusion, the alphabetical classification, recommended by the League of Nations, gives information about the nature of the groups.

The ABO groups are inherited according to Mendelian principles as shown by Bernstein (1924, 1925). A child gets from each parent one of three antigens A, B or O. O is more than merely an absence of A and B; it is itself an antigen, although a poor one. Antibodies to O can be produced by immunizing animals, and they occur spontaneously in some animal sera and very rarely in human serum. Anti-O is often and unfortunately called "alpha$_2$" because it reacts with most A_2 cells, probably the heterozygotes A_2O. The factors or genes which determine the heredity are carried on the chromosomes in the nuclei of the cells. In the spermatozoa and ova there are 24 chromosomes, which at fertilization pair with their opposite numbers to make the 24 pairs of chromosomes found in all cells of the body except the gametes; each of a pair is complementary to the other. As a child may acquire genes for A, B or O from each parent, the antigens it receives may be as shown in the Table:

TABLE II

Gene from one parent	Gene from other parent	Combination of genes [genotype]	Serologically demonstrable blood group [phenotype]
A	A	AA	A
A	O	AO	A
B	B	BB	B
B	O	BO	B
A	B	AB	AB
O	O	OO	O

When A or B is transmitted with O, only A or B is serologically demonstrable; A and B are both "dominant" to O, or O is "recessive" to the other two; A and B are equally dominant and both are manifest in the group AB. AA, the homozygote, and AO, the heterozygote, both belong to group A, and no serological method of differentiating the homozygote and the heterozygote has yet been devised. The position of BB and BO is similar. In any person, half the germ cells carry one factor and the other half carry the other; thus an AB person produces A and B gametes in equal numbers; AO produces O and A in equal numbers, and so on; whilst AA, BB and OO yield only germ cells of one sort —A, B or O. No child can have an antigen which is not present in one or other of his parents. The 3 pedigrees given below illustrate more clearly the method of inheritance:



Sub-groups of the ABO System

Dungern & Hirschfeld (1911) described the sub-groups A_1 and A_2, A_1B and A_2B. Nearly all anti-A sera (from group B donors) contain two anti-A agglutinins, alpha and alpha$_1$; alpha reacts with all the sub-groups A_1, A_2, A_1B and A_2B, while alpha$_1$ reacts only with A_1 and A_1B and *not* with A_2 and A_2B. To test for the sub-groups, alpha$_1$ is obtained free from alpha by absorbing alpha from a group B serum (chosen because it contains alpha$_1$ in good amount) with A_2 cells. Alpha$_1$ occurs naturally in the sera of some A_2 and A_2B people, and anti-O very rarely in some A_1, B and A_1B sera. A_2 reacts more weakly than A_1 and although it has been suggested that the difference between them is only quantitative, there is no doubt that it is qualitative. The sub-groups increase the number of groups from four to six: A_1, A_2, B, A_1B, A_2B and O. The inheritance of this extended system is explained by a simple extension of Bernstein's theory. Instead of the three original factors A, B and O, there are now four, A_1, A_2, B and O, with A_1, A_2 and B dominant to O, and A_1 dominant to A_2. A child gets one of the four factors from each parent and may be:

TABLE III

A_1A_1, A_1A_2, A_1O	all sub-group A_1	BB, BO	both group B
		A_1B	sub-group A_1B
A_2A_2, A_2O	both sub-group A_2	A_2B	sub-group A_2B
		OO	group O

Other more weakly reacting forms A_3, A_4 and A_5 have been described.

MN Blood Groups

Landsteiner & Levine (1928a) described another system of human blood groups depending on two antigens M and N, and according to which are in the cells, people can be divided into three groups M, MN and N, which have no connection at all with the ABO groups. Later (1928b) they showed that both M and N are inherited as characters without dominance, so that when they occur together in group MN they are both manifest; nobody lacking both M and N has yet been found, and there is therefore no group corresponding to the O group of the ABO system. A person of group M has received M from each parent, is homozygous MM and will produce only M gametes; group N is similar, homozygous NN. The heterozygote, MN, gets M from one and N from the other parent, and produces M and N gametes in equal numbers. If one parent of a mating is M, no N child, and if one parent is N no M child can result.

Anti-M and anti-N agglutinins do not occur naturally in human serum, though nine or ten cases of sera containing anti-M and one of anti-N have been reported. Testing for M and N is done with the sera of rabbits which have been immunized by the injection of suitable erythrocytes. Before the sera can be used it is necessary to remove the species antibodies, which will react with all kinds of human blood,

and leave only the specific anti-M or anti-N. Correct diagnosis of these groups requires very great experience.

Rh Factor

The latest factor, the Rh antigen (agglutinogen), was described by Landsteiner & Wiener (1940, 1941), who found that the serum of a rabbit or guinea-pig which had been injected with the erythrocytes of the Rhesus monkey agglutinated the cells of 85 % of white people in America and failed to agglutinate those of the other 15 %. The two classes are called Rh-positive and Rh-negative. The distribution is similar in Britain (Boorman, Dodd & Mollison, 1942; Hoare, 1943; Taylor & Race, 1944a), but there are marked racial differences. The proportion of positives is higher in American negroes, and Rh-negatives are very rare in some races; only one was found by Landsteiner, Wiener & Matson (1942) in 120 American Indians and one in 150 Chinese (Levine & Wong, 1943). Rh is inherited as a dominant character, the gene Rh determining its presence, and the gene rh its absence; its distribution is the same in the two sexes, and it seems to be quite independent of any other known blood-group antigen. It is present at birth.

Essentials of the Rh Factor

When the erythrocytes of a Rhesus monkey are injected into the circulation of a rabbit (or guinea-pig), the serum of the rabbit may acquire the property of agglutinating the erythrocytes of some (about 85 %) human beings, but not of others (about 15 %).

Erythrocytes of the Rhesus monkey contain an antigen which causes the production of an antibody (anti-Rh agglutinin) in the serum of the rabbit.

The antigen in human erythrocytes which reacts with the anti-Rh agglutinin is called the Rh factor (Rh for Rhesus).

The Rh factor is present in the erythrocytes of approximately 85 % of human beings. Such persons are described as Rh-positive, and their erythrocytes are agglutinated by sera which contain anti-Rh agglutinin.

The remaining 15 % are described as Rh-negative—that is, their erythrocytes do not contain the Rh antigen, and are therefore not agglutinated by sera containing anti-Rh agglutinin.

When blood from an Rh-positive donor (that is, a donor whose erythrocytes contain Rh antigen) reaches the circulation of an Rh-negative recipient, the serum of the recipient may be stimulated to produce Rh antibody (anti-Rh agglutinin). That is, the Rh-negative recipient may react to the erythrocytes of the Rh-positive donor as the rabbit reacts to the erythrocytes of the Rhesus monkey.

If Rh antigen has previously reached the circulation of an Rh-negative recipient (by transfusion or via the placenta from the child of an Rh-positive father), anti-Rh agglutinin may be present in the serum, and subsequent transfusion of Rh-positive blood may result in hæmolytic reactions.

Anti-Rh agglutinin which has been produced in maternal serum passes across the placenta, reacts with the Rh antigen in the erythrocytes of the Rh-positive child, and causes hæmolytic disease of the newborn (erythroblastosis fœtalis).

Before long, agglutinins identical with anti-Rh were found in the sera of certain persons who had hæmolytic reactions following transfusion of blood which, according to the ABO groups, was compatible. The recipients were all Rh-negative and had either been transfused before, or were women receiving a transfusion in connection with childbirth. The first group had been immunized by earlier transfusions of positive blood; the second by Rh antigen present in the fœtus and inherited from the father, the antigen having crossed the placenta from fœtus to mother. When transfusion reactions due to anti-Rh were found in the mothers of infants suffering from hæmolytic disease of the newborn (erythroblastosis fœtalis) the connection between immunization of the mother and this disease of the baby was quickly realized. According to Levine, Burnham, Katzin & Vogel (1941) it results from the isoimmunization of a mother by an erythrocyte antigen which she lacks, but which the child has inherited from the father, and the subsequent passage through the placenta of the resulting antibody to act on the susceptible fœtal blood. As 90 % of the mothers concerned are Rh-negative, and anti-Rh can be found in the sera of a large proportion of them, and as every affected child of such a mother tested has always been Rh-positive, the importance of Rh in the causation of this disease cannot be doubted.

Confirmation of the part played by Rh in the ætiology of hæmolytic disease of the newborn has been provided by many workers, and in Britain by Boorman, Dodd & Mollison (1942, 1944); Taylor (1943); Race, Taylor, Cappell & McFarlane (1943); Hoare (1943) and Langley & Stratton (1944). As soon as the importance of isoimmunization was understood, American workers began to treat affected infants by transfusion with Rh-negative blood, and Gimson (1943) has reported very encouraging results from a series so treated in London, whilst Mollison (1943) has followed the survival of the cells transfused into affected infants.

In the 10 % of cases where the mother is Rh-positive it seems that some other erythrocyte antigen must be responsible. Only anti-A and anti-B occur regularly in human sera under normal conditions; the sub-group antibodies alpha$_1$ and anti-O occasionally; and anti-M as a curiosity. Any of the above antigens, or some unknown and uncharted one, may possibly give rise to isoimmunization and cause a transfusion reaction or hæmolytic disease of the newborn. The recessive gene rh produces an antigen, and agglutinins which react with it have been made in mothers who lack it, by erythroblastotic babies who have got rh from their fathers, and it is possible that quite a proportion of cases with Rh-positive mothers are caused by this type of agglutinin.

Rh Sub-types

The Rh factor is not as simple as was at first supposed. Wiener (1942) found a human serum which agglutinated only about 70 % of bloods (85 % were positive with the other human and immune animal sera). Rh-negative bloods did not react with the new serum and the 70 % which did were said, by analogy with the sub-groups of A, to be of the sub-type Rh_1, whilst the 15 % or so positive with the original and negative with the new serum, were of the sub-type Rh_2, as shown in Table IV.

Wiener (1942) referred to a peculiar agglutinin in the serum of an Rh_1 mother of an erythroblastotic infant. The serum, found by Levine, Javert and Katzin, was said to react with Rh-negative and Rh_2 bloods, that is, with about 30 % in all. Other accounts of this serum, named anti-Hr, because it worked, as it were, the other way round from anti-Rh, give different percentages of reactions and suggest that it was troublesome to work with. A similar, but not necessarily identical serum, called St from the first two letters of the mother's surname, has been investigated by Race & Taylor (1943), and details of its discovery and of the clinical condition of the Rh-positive donor and her erythroblastotic baby have been given by McCall, Race & Taylor (1944). St reacts with the blood of all Rh-negative and all heterozygous Rh-positive persons (Rhrh), who are recognized by being Rh-positive parents or children of Rh-negative subjects, but it fails with about 20 % of bloods, all of which must be homozygous (RhRh) and represent about half of the Rh-positive homozygotes (about 38 % of the population).

TABLE IV

Erythrocytes	85 % human or animal serum	70 % serum (anti-Rh_1)
Rh_1 (70 %)	+	+
Rh_2 (15 %)	+	−
Rh-negative (15 %)	−	−

To explain the sub-types Rh_1 and Rh_2, Wiener (1942) suggested that instead of the original genes Rh and rh, there were three allelomorphs Rh_1, Rh_2 and rh governing the heredity of the Rh factors. In a system of n allelomorphs the number of genotypes is given by $\frac{n(n+1)}{2}$. Thus Rh_1, Rh_2 and rh produce six genotypes, and if the frequencies of the genes are $\overline{Rh_1} + \overline{Rh_2} + \overline{rh} = 1{\cdot}0$,* then the frequencies of the six genotypes are given by the terms of

$$(\overline{Rh_1} + \overline{Rh_2} + \overline{rh})\,(\overline{Rh_1} + \overline{Rh_2} + \overline{rh}).$$

Race & Taylor (1943) suggested that the 20 % of cells which were St-negative were of the genotype Rh_1Rh_1. By taking the square roots of 0·20 and of 0·15, values of 0·45 and 0·39 are obtained for the frequencies of the genes Rh_1 and rh, leaving 0·16 for that of Rh_2. The percentages of the six genotypes calculated from these frequencies are given in Table V.

Striking confirmation of the belief that St-negative cells are Rh_1Rh_1 is provided by these genotype frequencies: (i) Those containing Rh_1 total 69·5 %, a figure in remarkable agreement with the 70 % of reactors with Wiener's anti-Rh_1 serum (the sub-type Rh_1). (ii) The genotypes Rh_2Rh_2 and

* [The bar over the gene indicates the frequency of the gene as opposed to the gene itself, e.g., the frequency of the gene Rh is written $\overline{Rh}$.]

TABLE V

Phenotype	Genotype	% (approx.)	Frequency
Sub-type Rh_1 . .	Rh_1Rh_1	20·0	$\overline{Rh_1}^2$
	Rh_1Rh_2	14·4	$2\overline{Rh_1Rh_2}$
	Rh_1rh	35·1	$2\overline{Rh_1rh}$
Sub-type Rh_2 . .	Rh_2Rh_2	2·6	$\overline{Rh_2}^2$
	Rh_2rh	12·5	$2\overline{Rh_2rh}$
Rh-negative . .	rhrh	15·0	$\overline{rh}^2$

Rh_2rh total 15·1 % and are the sub-type Rh_2 which Wiener had found to be 15 %.

Shortly after the discovery of St serum, Race, Taylor, Boorman & Dodd (1943) found another type of anti-Rh serum from the mother of an erythroblastotic infant. This serum agglutinated about 30 % of bloods, but failed to agglutinate Rh-negatives. The three genotypes in Table V which contain Rh_2 total 29·5 %, and it was soon realized that the latest serum reacted with Rh_2. A similar anti-Rh_2 serum was described by Wiener (1943a). Within a week or two, Race, Taylor, Cappell & McFarlane (1944) identified a serum as anti-Rh_1, and it was then possible to test cells with the four different types of serum. The reactions caused by the three genes are:

TABLE VI

	Antisera			
	Anti-Rh	Anti-Rh_1	Anti-Rh_2	St
Rh_1 . .	+	+	−	−
Rh_2 . .	+	−	+	+
rh . . .	−	−	−	+
and by the six genotypes:				
Rh_1Rh_1 . .	+	+	−	−
Rh_1Rh_2 . .	+	+	+	+
Rh_1rh . .	+	+	−	+
Rh_2Rh_2 . .	+	−	+	+
Rh_2rh . .	+	−	+	+
rhrh . .	−	−	−	+

Although it includes the most important part, the above description of the three antigens Rh_1, Rh_2, and rh and the reactions given by the four types of antiserum is not the whole story of the Rh antigens and antisera. Besides the three common allelomorphs Rh_1, Rh_2 and rh, four rare ones have been found, Rh′, Rh″, Rh_o, each having a frequency of 1 % or less, and Rh_y, which is very rare indeed (Wiener, 1943b; Race, Taylor, Cappell & McFarlane, 1944; Race & Taylor, 1944). With seven allelomorphs there are 28 genotypes. The six common ones in Table V are the genotypes of about 93 % of people, and the remaining 22 genotypes cover the other 7 %. Each Rh antiserum mentioned so far contains one agglutinin: (i) The 85 % reacting serum of Table IV, anti-Rh, contains one agglutinin which by analogy with the use of the Greek alpha and beta for the anti-A and anti-B of the ABO groups may be called rho. (ii) Anti-Rh_1 contains rho_1, and (iii) anti-Rh_2 has rho_2. But sera containing mixtures do occur; thus anti-Rh′ has rho + rho_1 and, anti-Rh″ has rho + rho_2. Details are given in the following Table:

TABLE VII

Antiserum	Percentage of people positive	Agglutinins	Reacts with Antigens				
			Rh_1	Rh′	Rh_2	Rh″	Rh_o
Anti-Rh .	85	rho	+	−	+	−	+
Anti-Rh_1 .	70	rho_1	+	+	−	−	−
Anti-Rh_2 .	30	rho_2	−	−	+	+	−
Anti-Rh′ .	87	rho + rho_1	+	+	+	−	+
Anti-Rh″ .	85 +	rho + rho_2	+	−	+	+	+

St reacts with about 80 % of people, including all rhrh; it also reacts with rh and Rh_2 wherever they occur, thus:

TABLE VIII

	Rh_1	Rh′	Rh_2	Rh″	Rh_o	rh	Rh_y
St serum	−	−	+	+	+	+	−

Apart from its being negative with St and positive with rho_2, the reactions of Rh_y are not yet known.

Stratton (1944) has described a maternal serum which contains the three agglutinins rho, rho_1 and rho_2, and the authors of this article are aware of the occurrence of another serum of this sort.

The terminology used in the present account of the Rh system of blood groups will certainly not be permanent. Race (1944) has described a most ingenious and attractive scheme, formulated by R. A. Fisher, for the Rh genes, antigens and antibodies. The scheme postulates the existence of an eighth allelomorph, Rh_z, and two further agglutinins both different from St, but like St reacting with rh. The terminology of the scheme differs markedly from any used so far.

In seeking Rh-negative bloods for transfusion, those not agglutinated by 85 % anti-Rh serum should be further examined with sera containing rho_1, and, if available, rho_2, otherwise the rare genotypes Rh′Rh′, Rh′rh, Rh″Rh″, Rh″rh and Rh′Rh″ will be diagnosed as Rh-negative and may, on transfusion into a person with a corresponding agglutinin, cause a hæmolytic reaction. About half the human sera are anti-Rh′, so that the use of the rare Rh′ type as negative blood is very risky. With Rh″ the risk is much less, as sera having rho_2 are very rare. By using an anti-Rh′ serum for routine testing of donors almost all the risk would be eliminated. When persons of the five Rh′ and Rh″ types are transfused it will usually be safest to give Rh-negative blood. The very infrequent Rh′Rh′ may, however, make St antibody for which negative blood is incompatible. These possibilities emphasize the value of direct matching of the recipient's serum and intended donor cells in a tube at 37° C. These rare genotypes have increased the number of Rh-positives from 85 to 87 % and have reduced the complete negatives to 13 %.

In Table IX are set out the reactions given by the antigens and the various antisera, and in Table X are the phenotypic combinations, the interpretation in terms of genotypes, and the approximate frequencies.

TABLE IX

Antigens	Frequency (%)	Antisera					
		Rh_1	Rh_2	St	Rh′	Rh″	
Rh_1 . . .	0·43	+	+	−	−	+	+
Rh_2 . . .	0·15	+	−	+	+	+	+
Rh_o . . .	0·01 or less	+	−	−	+	+	+
Rh′ . . .	,,	−	+	−	−	+	−
Rh″ . . .	,,	−	−	+	+	−	+
rh . . .	0·36	−	−	−	+	−	−
Rh_y . . .	very rare	?	?	+	−	?	+

The reactions with anti-Rh′ and anti-Rh″ sera, included in the Tables for completeness, do not add anything to the information given by the other four sera. It will be seen that the majority of genotypes are not completely isolated, but that in most groups there is one very much more frequent than the rest.

Serological tests do not differentiate between Rh_1Rh_2 and Rh_2rh, but the genotypes of about 80 % of people can be determined (Race, Taylor, Cappell & McFarlane, 1944), ignoring the rare ones, and some of these can be inferred from family investigations. In about three cases out of four, serological tests show whether an Rh-positive person is homozygous RhRh or heterozygous Rhrh, and are of great prognostic importance in families in which erythroblastosis fœtalis has occurred (Taylor & Race, 1944b). Knowledge of the genotypes should increase the usefulness of the Rh group in linkage studies.

TABLE X

Antigens	Rh	Rh_1	Rh_2	St	Rh′	Rh″	Frequency (approx.) (%)
Rh_1Rh_1	+	+	−	−	+	+	19
Rh_1Rh'	+	+	−	−	+	+	Rare
Rh_1Rh_2	+	+	+	+	+	+	13
Rh_1Rh''	+	+	+	+	+	+	Rare
$Rh'Rh_2$	+	+	+	+	+	+	Rare
Rh_2Rh_2	−	−	+	+	−	−	2
Rh_2rh	−	−	+	+	−	−	12
Rh_2Rh''	+	−	+	+	+	+	Rare
Rh_2Rh_0	+	−	+	+	+	+	Rare
Rh_0Rh''	+	−	+	+	+	+	Very rare
Rh_1rh	+	+	−	−	−	−	33
Rh_1Rh_0	+	+	−	+	+	+	Rare
$Rh'Rh_0$	+	+	−	+	+	+	Very rare
Rh_0Rh_0	+	−	−	+	+	+	Very rare
Rh_0rh	+	−	−	+	+	+	Rare
rhrh	−	−	−	+	−	−	13
Rh′rh	−	+	−	+	+	−	Rare
Rh″rh	−	−	+	+	−	+	Rare
Rh″Rh″	−	−	+	+	−	+	Very rare
Rh′Rh″	−	+	+	+	+	+	Very rare
Rh′Rh′	−	+	−	−	+	−	Very rare
Rh_1Rh_y	+	+	+	−	+	+	Very rare

Other Rh_y genotypes are very rare and have doubtful reactions.

Genetic and Forensic Implications

The importance of blood groups is not confined to their relationship to transfusion. As they provide markers for the chromosomes concerned with their inheritance, they are of interest to the geneticist. For the genes of another hereditary character may be on the same pair of chromosomes as those of a system of blood groups, and the transmission of the two may be related. ABO and MN tests are widely used in cases of disputed parentage and other medico-legal grouping tests are made on blood and stains. The six A_1A_2BO and the three MN groups furnish 18 blood-group types and with the 11 Rh divisions of Table X enable the recognition of 18×11 (198) different types. Other factors have been found, but because of technical difficulties or because the antibodies are not easily obtained, they are not yet widely used. Anti-P from normal or immune animal sera and occasionally from human sera (Landsteiner's extra agglutinin No. 1) differentiates P (a dominant character) and non-P persons.

The ABO antigens are not confined to erythrocytes, but occur in tissues and various body fluids. Many workers have thought that M, N and Rh antigens do not exist outside the erythrocytes, but Boorman & Dodd (1943) have confirmed the report of Kosjakov & Tribulev (1940) that M and N do, and in addition have found Rh in tissues and saliva.

People can also be divided into two types according to whether the saliva contains in considerable amount the antigens of the ABO group to which they belong (secretors), or lacks them almost entirely (non-secretors). In secretors the antigens are in most other body fluids besides saliva. The ability to secrete A, B or O is a dominant character and its inheritance is quite independent of that of the groups themselves. It is not known whether the gene which controls secretion of ABO antigens governs the secretion of other antigens, e.g. Rh, or even whether the presence of Rh in saliva is an inherited character. The genes for the various blood groups and secretion appear to be on different chromosomes and so provide linkage markers for five (excluding sex) of the 24 pairs of chromosomes, whilst the characters themselves permit the division of mankind into a very large number of types thus:

TABLE XI

$$\underset{6}{\text{ABO}} \times \underset{3}{\text{MN}} \times \underset{11}{\text{Rh}} \times \underset{2}{\text{P}} \times \underset{2}{\text{Secretion}} = 792 \text{ types}$$

Ability to recognize more Rh genotypes or the discovery of other antigens would greatly increase the number of types, and Landsteiner suggested the possibility of establishing the individuality of blood just as can be done with finger-prints.

The proportion of people in the types varies enormously; some are very rare. There are great racial differences which are of interest to the anthropologist (see Table XII).

TABLE XII

Britain %	O 46	A 42	B 9	AB 3	But marked differences in different parts.
India %	O 30	A 24	B 37	AB 8	Very great differences in different parts.
Most places %	M 30	MN 50		N 20	Occasionally a race has little of one or other antigen.

	P	Non-P	Secretor	Non-secretor
America % (whites)	75–80	20–25	75–80	20–25

Rh has been mentioned above, and evidence of marked racial differences in the distribution of the Rh genes is being obtained.

REFERENCES

Bernstein, F. (1924) *Klin. Wschr.* **3**, 1495
Bernstein, F. (1925) *Z. indukt. Abstamm.- u. VererbLehre*, **37**, 237
[1] Boorman, K. E. & Dodd, B. E. (1943) *J. Path. Bact.* **55**, 329
[2] Boorman, K. E., Dodd, B. E. & Mollison, P. L. (1942) *Brit. med. J.* **2**, 535 & 569
[3] Boorman, K. E., Dodd, B. E. & Mollison, P. L. (1944) *J. Obstet. Gynæc.* **51**, 1
Dungern, E. von, & Hirschfeld, L. (1911) *Z. ImmunForsch.* **8**, 526
[4] Gimson, J. D. (1943) *Brit. med. J.* **2**, 293
[5] Hoare, E. D. (1943) *Brit. med. J.* **2**, 297
Janský, J. (1907) *Sborn. klin. Praha*, **8**, 85; résumé in French in *J. Immunol.* 1931, **20**, 136
Kosjakov, P. N. & Tribulev, G. P. (1940) *J. Immunol.* **37**, 297
Landsteiner, K. (1900) *Zbl. Bakt.* **27**, 357
Landsteiner, K. & Levine, P. (1928a) *J. exp. Med.* **47**, 757
Landsteiner, K. & Levine, P. (1928b) *J. exp. Med.* **48**, 731
Landsteiner, K. & Wiener, A. S. (1940) *Proc. Soc. exp. Biol. N.Y.* **43**, 223
Landsteiner, K. & Wiener, A. S. (1941) *J. exp. Med.* **74**, 309
Landsteiner, K., Wiener, A. S. & Matson, G. A. (1942) *J. exp. Med.* **76**, 73
[6] Langley, F. A. & Stratton, F. (1944) *Lancet*, **1**, 145
Levine, P., Burnham, L., Katzin, E. M. & Vogel, P. (1941) *Amer. J. Obstet. Gynec.* **42**, 925
Levine, P. & Wong, H. (1943) *Amer. J. Obstet. Gynec.* **45**, 832
[7] McCall, A. J., Race, R. R. & Taylor, G. L. (1944) *Lancet*, **1**, 214
[8] Mollison, P. L. (1943) *Arch. Dis. Childh.* **18**, 161
Moss, W. L. (1910) *Johns Hopk. Hosp. Bull.* **21**, 63
[9] Race, R. R. (1944) *Nature*, **153**, 771
Race, R. R. & Taylor, G. L. (1943) *Nature*, **152**, 300
Race, R. R. & Taylor, G. L. (1944) *Nature*, **153**, 560
Race, R. R., Taylor, G. L., Boorman, K. E. & Dodd, B. E. (1943) *Nature*, **152**, 563
[10] Race, R. R., Taylor, G. L., Cappell, D. F. & McFarlane, M. N. (1943) *Brit. med. J.* **2**, 289
Race, R. R., Taylor, G. L., Cappell, D. F. & McFarlane, M. N. (1944) *Nature*, **153**, 52
Stratton, F. (1944) *Nature*, **153**, 773
[11] Taylor, G. L. (1943) *Proc. roy. Soc. Med.* **36**, 225
Taylor, G. L. & Race, R. R. (1944a) *Med. Pr.* **211**, 86 & 104
[12] Taylor, G. L. & Race, R. R. (1944b) *Brit. med. J.* **1**, 288
Wiener, A. S. (1942) *Amer. J. clin. Path.* **12**, 302
Wiener, A. S. (1943a) *Science*, **98**, 182
Wiener, A. S. (1943b) *Proc. Soc. exp. Biol. N.Y.* **54**, 316

[1] [*see BMB* **438**] [2] [*see BMB* **8**] [3] [*see BMB* **432**]
[4] [*see BMB* **427**] [5] [*see BMB* **428**] [6] [*see BMB* **430**]
[7] [*see BMB* **431**] [8] [*see BMB* **429**] [9] [*see BMB* **435**]
[10] [*see BMB* **426**] [11] [*see BMB* **85**] [12] [*see BMB* **433**]

SOME RECENT OBSERVATIONS ON THE INHERITANCE OF BLOOD GROUPS

R. R. RACE, M.R.C.S., L.R.C.P.

The statistical methods and the appropriate family investigations necessary to show how blood-group antigens are inherited slowly emerged from the work on the ABO and MN systems. Consequently, when the Rh groups were discovered by Landsteiner and Wiener, this familiarity with the problem contributed to the fairly rapid recognition of seven allelomorphic forms of the gene responsible.

The A_1A_2BO genes occupy a single locus, and so do the MN genes, and it was generally supposed that there was but one locus for the seven alternatives of the Rh gene. Early in this year, however, in order to correlate the three categories antigens, genes or allelomorphs, and antibodies, Fisher (Race, 1944) suggested that either each gene is associated with three antigens, or that three closely linked loci are involved. Each of Fisher's three antigens has two alternatives and these three pairs of two alternatives make eight possible combinations in the gamete. The hypothesis thus predicts the discovery of an eighth allelomorph.

Landsteiner & Wiener (1941), in the earliest family investigations, demonstrated that the Rh gene was not sex-linked nor partially sex-linked, and that it must therefore be carried on one of the 23 autosomes. Linkage was looked for, but not detected, between the Rh gene and the ABO and MN genes. So far no results have been published of any search for linkage between the Rh gene and a disease gene, nor between it and the remaining two common recognisable genes—those responsible for tasting phenyl-thiocarbamide and for secreting the ABO antigens in the saliva.

It has been known for some time that gypsies of Hindu origin, who have lived in Hungary for more than 200 years, have the modern Hindu distribution of the ABO groups; and that German colonists who settled in Hungary some 250 years ago, and did not intermarry with the Hungarians, have the modern German distribution. Both these distributions are quite unlike that of the Hungarians. Early in the present war the frequency of the ABO groups amongst donors in the United Kingdom was analysed by Fisher & Taylor (1940); the Scottish and North Irish frequencies were like those found in Iceland, but quite unlike the continental and South English distribution. This suggests that these northern frequencies may have been much the same since Viking times. Further, Hart (1944) finds that in the rural population of Ulster the donors with English names have the English distribution of groups although it is more than 400 years since their ancestors settled there.

Thus, when race-crossing is negligible, the proportions of the ABO groups probably remain constant over long periods of time. Consequently Ford (1942) considers these groups an example of a balanced polymorphism. This state entails, as shown by Fisher (1927), a balance of selective influences. In the case of these groups, selection may work on some unknown ancillary effect of the genes.

There is so far no clear evidence of the balanced nature of the polymorphism due to Rh. Indeed it has been argued by Haldane (1942) that the proportions of these groups in the white population must be changing fairly rapidly, owing to the constant selection against the heterozygote, Rhrh, exerted by hæmolytic disease of the newborn. Such adverse selection would tend to bring to a very low level the rarer of the two genes —rh. The relatively high proportion of Rh-negatives amongst the whites is probably due, according to Haldane, to their having originated from a crossing of two races, one mostly Rh-positive (like the modern Chinese) and the other predominantly Rh-negative. Haldane therefore considers that the gene ratio amongst whites is highly unstable and that rh is in the process of being eliminated, the present polymorphism being of a transient type. Hogben (1943), on the other hand, thinks that the ratios are constant, and that the selection against the gene rh is counterbalanced by a high mutation rate to rh. The rate of mutation would have to be many times higher than any yet known.

Fisher has suggested another way in which the gene frequency of rh may be maintained in spite of hæmolytic disease. In human families deaths in infancy are usually associated with an increased number of births. In hæmolytic families, even with homozygous fathers, where every child is liable to the disease, not all are killed by it; in such cases additional births will have partially compensated for those lost. With heterozygous fathers, on the other hand, half the children will be recessives, like their mothers, and these will have the higher rate of survival, so that even a partial replacement of children lost may over-compensate the loss of rh genes.

There is then no solid ground for assuming that the rh gene is being eliminated, even if, as is still doubtful, Rh-negative women have on the average slightly fewer surviving children than Rh-positive women. The physiological and psychological tendencies to replace children lost early in life are illustrated in a series of families of hereditary acholuric jaundice collected by the writer (Race, 1942) when at the Galton Laboratory. It was then found that affected females had an exceptionally large number of stillbirths and infantile deaths, but, nevertheless, had a *larger* number of surviving children than their normal sisters.

In this case, still-births and neonatal deaths were a stimulus to over-compensation, and it is to be expected that mothers of children with hæmolytic disease, whose husbands are heterozygotes, Rhrh, may eventually produce more than their share of Rh-negative children.

Haldane (1942) has discussed the eugenic aspect of the Rh-rh gene substitution, and points out that, although it is responsible for more deaths than any other known human gene substitution, it is obviously going too far to recommend that Rh-negative women should marry exclusively Rh-negative men. The Rh-positive babies of only about 5% of Rh-negative women are affected with hæmolytic disease. Such women may possess highly permeable placentæ, and if the abnormal permeability was due to a gene and the gene could be recognised, then there would be a strong case for discouraging a mating which involved (i) an Rh-negative woman and (ii) this gene for placental permeability and (iii) an Rh-positive man.

REFERENCES

Fisher, R. A. (1927) *Trans. roy. ent. Soc. Lond.* **75**, 269.
Fisher, R. A. & Taylor, G. L. (1940) *Nature*, **145**, 590
Ford, E. B. (1942) *Genetics for medical students*, London, p. 117
Haldane, J. B. S. (1942) *Ann. Eugen., Camb.* **11**, 333
Hart, E. W. (1944) *Ann. Eugen., Camb.* **12**, 89
Hogben, L. (1943) *Nature*, **152**, 721
Landsteiner, K. & Wiener, A. S. (1941) *J. exp. Med.* **74**, 309
[1] Race, R. R. (1942) *Ann. Eugen., Camb.* **11**, 365
[2] Race, R. R. (1944) *Nature*, **153**, 771

[1] [*see BMB* 437]

[2] [*see BMB* 435]

OCCURRENCE AND NATURE OF HUMAN BLOOD-GROUP SUBSTANCES

W. T. J. MORGAN, Ph.D., D.Sc.

Rather more than 40 years have passed since Landsteiner and his pupils announced that human bloods can be divided into four serological groups. Although the modern technique of blood transfusion that has been evolved from this important discovery is now to be regarded as an established and safe routine medical procedure, it is unfortunately not yet possible to record similar outstanding advances in what may be considered to be the fundamental aspect of the subject, namely,

the chemical nature of the substances that are directly responsible for the strict specificity of tissue cells and tissue fluids.

Many workers have at one time or another attempted to isolate the blood-group antigens from human erythrocytes but it may be said that, in general, these attempts have failed. The work has proceeded mainly along two lines: the extraction of the cells or stromata with simple aqueous reagents, or with organic solvents. In no instance has the isolation of any of the blood-group antigens from erythrocytes been recorded but, on the other hand, substances have often been isolated which, though devoid of antigenic power, have shown intense blood-group specificity.

Alcohol-Soluble Blood-Group Substances

The earliest observations dealing with this aspect of the subject were those of Schiff & Adelsberger (1924) and of Landsteiner & van der Scheer (1925), who obtained non-antigenic group-specific substances by extraction of erythrocytes with alcohol. Hallauer (1934), using a similar technique, described the isolation of specific non-antigenic substances from all three (A, B, O) blood groups. Apart from the conclusion based on a few qualitative tests that the specific substances are largely carbohydrate, the chemical nature of these immunologically active factors was not determined. The composition of the specific material isolated by Hallauer, which is very similar for each specific blood group examined, was 43–46% C, 7–8·5% H, 6·8–7·0% N, 15–21% P. The high P content, if present as P in organic combination, is of considerable interest but, so far, no further details have been given and no confirmation of these important observations has been forthcoming. More recently Stepanov, Kusin, Makajeva & Kosjakov (1940) have also described the isolation of specific group substances from erythrocytes by means of alcoholic extraction. Although these substances give strong *in vitro* reactions with the homologous immune-serum produced by means of the corresponding erythrocytes or stromata, the alcohol-soluble material is itself devoid of antigenic properties.

Water-Soluble Blood-Group Substances

The occurrence of the blood-group substances in a water-soluble form in the tissue fluids and secretions of the body has been known for many years and an examination of the extensive literature on the subject shows that an insight into the chemical nature of the specific A, B and O group substances has been attained almost entirely through studies on these water-soluble group components.

Blood Group A-substances from Peptone, Pepsin and Gastric Mucin from the Pig

An early observation of Schiff (1930) revealed that commercial peptone contains a substance which inhibits the hæmolysis of sheep erythrocytes by an immune-serum to human erythrocytes belonging to group A. The isolation and characterisation of the specific group A-substance from peptone was reported by Goebel (1938), who showed that the substance is largely composed of a polysaccharide which is rich in glucosamine. The purest specimens obtained, however, still showed a weak biuret reaction indicating the presence of protein or of some kind of amino-acid complex. The A-substance contained 45–46% C, 6–7% H, 5–6% N and 9–10% acetyl, and showed a weak dextro-rotation $[\alpha]_{5461} + 10°$. An A-substance, probably identical with the A-substance obtained from peptone, has been isolated from commercial pepsin by Landsteiner & Harte (1940), and by Meyer, Smyth & Palmer (1937) as the result of their work on the glyco-proteins that are present in the gastric mucosa of the pig; these workers isolated from this tissue a polysaccharide which possesses intense A-activity and which contains *d*-galactose and N-acetylglucosamine in equimolecular quantities. Stacey (1943) has stated that the A-substance isolated from commercial pepsin contains *d*-mannose and *l*-fucose in addition to *d*-galactose and N-acetylglucosamine, but details of this work have not yet been published. The frequency with which the purified A-substance obtained from different sources was found to give a positive test for protein or for single amino-acids indicated that an amino-acid complex might be a true component of the specific A-substance. Landsteiner & Harte (1940) followed this clue by examining with more care the non-hexosamine nitrogenous constituents, and found that the products of acid hydrolysis of the A-substance contained 35–40% of the total nitrogen in the form of amino-acids; arginine, histidine and possibly alanine were identified by simple colour reactions. The analytical figures found for the composition of the A-substance isolated from peptone, pepsin and gastric mucin of animal origin are almost identical. It seems probable that the A-substance in peptone is derived from the pepsin used in its preparation. Similarly the A-activity shown by commercial pepsin is most probably due to its content of A-substance derived from pig gastric mucin.

In most of the earlier work the activity of the A-substance isolated from different sources was determined by means of the hæmolytic inhibition test, with the result that the destruction of an important serological property of the native A-substance, the power to inhibit A-isoagglutination, was overlooked. Landsteiner & Harte (1940) observed that treatment of the crude A-substance with formamide at 150° C. for 1 hour according to a technique introduced by Fuller (1938) for the isolation of bacterial polysaccharides, gave rise to a purified A-substance which showed only a fraction of its original power to inhibit isoagglutination, whereas its capacity to inhibit the hæmolysis of sheep cells by an anti-A rabbit serum was actually increased beyond the original value. These and other observations indicated that a carefully controlled isolation procedure must be used if the blood-group complexes are to be obtained in their "native" condition, that is, with their chemical, physical and immunological properties unchanged. Fortunately, considerable experience with a closely related problem, the isolation of certain labile bacterial antigens (Morgan, 1937; Morgan & Partridge, 1939, 1940, 1941, 1942) became available a few years ago, and the application of this knowledge has enabled methods to be elaborated whereby the specific group A-substance can be isolated from commercial pepsin and crude gastric mucin and yet retain its original power to inhibit A-isoagglutination largely unimpaired (Morgan & King, 1943). The A-substance prepared by these methods is similar in composition to that described by Landsteiner & Harte (1940), but differs from it in a number of important physical and immunological properties. The material shows not only a high serological activity as determined by the inhibition of isoagglutination but also the high viscosity that is so characteristic of native gastric mucin. Furthermore a 1% solution of the A-substance possesses the property of forming an elastic gel on the addition of borate buffer at *p*H 8·5. This property is readily lost on heating the A-substance in neutral, acid or alkaline solution at 100° C. for a few minutes, and it has been found that the addition of the buffer to a 1% solution of the A-preparation reveals at once, according to the formation or otherwise of the elastic gel, whether the material has been degraded during the course of its preparation. In addition to the rapid decrease in the viscosity of the A-substance that is brought about by heating solutions of the substance, there occurs at the same time a steady fall in the ability of the preparation to inhibit the isoagglutination of the A-cells by natural human anti-A (α) agglutinin. In order to avoid these irreversible changes it is essential that only the most gentle methods of isolation should be employed.

The undegraded A-substance isolated by Morgan & King (1943) from crude pig gastric mucin shows two other important properties that are absent from material prepared by methods that involve heating the mucin or pepsin in formamide or in dilute sodium carbonate. One characteristic property retained by the undegraded A-substance, which is itself without antigenicity, is its power to combine with the protein component of the somatic O antigen of *Bact. shigæ* to form an antigenic complex that gives rise to extremely potent and specific anti-A immune-body in the rabbit (Morgan 1941, 1943). The other property shown by the undegraded A-substance is its capacity to function as a bacterial "virulence enhancing" agent in the same manner as the crude gastric mucin of the pig. Both these properties are readily lost if the A-substance is heated or treated in any way that will destroy its viscosity or its capacity to inhibit A-isoagglutination, even when the power of the resulting material to inhibit the hæmolysis of sheep cells by anti-A serum is completely retained. The retention of these important properties of the native A-substance by carefully purified preparations completely justifies the extra trouble involved in the isolation of the blood-group substance.

The undegraded substance shows a high viscosity, η, 2·8

at a concentration of 0·5 % in saline. A typical analysis of the substance gave 45 % C, 6·5 % H, 6·0 % N and 10 % CH_3CO, and the material was found to be essentially homogeneous when examined at *p*H 4·0 or 8·0 in the Tiselius electrophoresis apparatus. Hydrolysis of the A-substance with 6N HCl gives rise to about 50 % reducing sugars, 27 % hexosamine, 4·5 % α-amino N (Van Slyke), and 2·2 % α-amino acid N. Treatment of the active material with 0·1 N sodium carbonate at 100° C. causes the complex to break up in such a manner that part of it will readily diffuse through a cellophane membrane while about two-thirds of the total material remains indiffusible. The amino-acid components are almost completely retained by the membrane and are still associated with a carbohydrate structure. The indiffusible complex, $[\alpha]_{5461} - 20°$, is practically non-reducing, is electrophoretically homogeneous and possesses less than 1 % of the original serological activity. The diffusate, however, shows strongly reducing properties without further acid hydrolysis and gives an immediate colour with Ehrlich's reagent, thus indicating the presence of an oxazole structure which has most probably been formed by the action of the alkali on an N-acetyl hexosamine molecule that possesses a free reducing group at C atom 1 (Morgan & Elson, 1934; Morgan, 1936). The alkali-labile linkages in the original A-substance are presumably a part of those glycoside linkages that join the C atom 1 of the N-acetylhexosamine molecules to other components of the A-complex. The diffusible material contains a considerable amount of N that is not accounted for as hexosamine-N or α-amino acid N. The results so far obtained seem to indicate that some but not all of the N-acetylhexosamine molecules contained within the A-substance are joined at C atom 1 to the amino-acid components of the serologically active complex.

A preliminary qualitative examination of the amino-acids present in the products of acid hydrolysis of the A-substance has been made by means of the chromatographic method described by Consden, Gordon & Martin (1944). At least 15 amino-acids are present as components of the A-complex and it seems probable that threonine and hydroxyproline are present in higher concentrations than are normally found in proteins. Cystine appears to be absent. The isolation of threonine from A-substance has been described by Freudenberg, Walsh & Molter (1942).

Blood-Group Substances from Human Sources

The difficulty of obtaining sufficient group-specific substances from human erythrocytes for detailed immunochemical studies has forced workers in this field to consider human tissue fluids and secretions as a source of the specific group substances. The capacity of certain individuals to secrete in their tissue fluids substances that possess a serological specificity very similar to or identical with their specific blood-group substance is now generally recognised. Those individuals who secrete their blood-group substances in a water-soluble form in the tissue fluids are termed " secretors," whereas those who do not secrete the active factors are called " non-secretors." It has been established that the presence or absence of water-soluble specific blood-group substances in the secretions and tissue fluids of an individual is controlled by a single gene S, dominant in effect, which allows the secretion of the specific substance. There is no evidence of linkage between the character " secretor " and any of the A, B and O blood groups.

Urine: As a result of the detection of the specific blood-group substances in human urine by Yosida (1928), several early attempts were made to isolate the specific substances from this source, but apart from demonstrating that the active material is largely polysaccharide in nature and contains *d*-galactose and N-acetylglucosamine, little progress was made in determining the chemical nature of the group substances. Indeed, it was not possible to differentiate chemically between substances isolated from the urine of individuals belonging to groups A, B or O. A serious disadvantage in using urine as a source of the specific substance is its very small content of the serologically active factor, and work on urine as a starting material has now been largely abandoned.

Gastric juice: Witebsky & Klendshoj (1940) reported the isolation of the blood-group B substance from human gastric juice but again, apart from showing that the material isolated possessed strong B-specific serological activity and gave rise to 75 % of reducing sugars on acid hydrolysis, the results gave little information concerning the chemical properties of this important substance. Material, 13·5 mg., containing about 1·5 % N was isolated from 110 ml. of gastric juice. Witebsky & Klendshoj (1941) have also described the isolation of a specific O-substance from human gastric juice. Owing to lack of material, detailed chemical examination of the specific substances was not possible.

Saliva: Specific blood-group substances have been obtained from human saliva by Landsteiner & Harte (1941) who recovered about 15 mg. of active substance from 500 ml. portions of the secretion. On a dry-weight basis, the substances were at least 50 times as active as the total solids in the saliva. The preparations made from the saliva from " secretors " belonging to groups A, B and O showed little difference chemically. The materials contained about 5·5 % N, 2·5 % α-amino-acid N, 21–23 % hexosamine and gave 45–48 % reducing sugar after acid hydrolysis. The Millon test for tyrosine, Ehrlich test for tryptophane with *p*-dimethylaminobenzaldehyde, and the lead test for sulphur were negative. Distinct reactions were obtained with diazotized sulphanilic acid and for arginine by means of Sakaguchi's test.

Pseudo-mucinous ovarian cyst fluids: The concentration of the active factors in saliva and gastric juice is high when compared with that of many other tissue fluids and secretions, but even here the active substance represents only a small part of the total solid matter of the secretions, which are, moreover, difficult to obtain in useful quantities. In certain instances, however, mucilaginous fluids accumulate in man as a result of pathological changes or of tissue overgrowth, and the knowledge that pseudo-mucinous ovarian cysts are of frequent occurrence prompted Morgan & van Heyningen (1944) to examine the fluid contents of these pathological overgrowths for blood-group substances. An examination of 50 pseudo-mucinous cysts revealed that, when these cyst fluids were obtained from women who possess the power to secrete their specific blood-group substance in a water-soluble form, the fluids are a convenient and potent source of the group-specific substances A, B and O. Cyst fluids vary in volume from 100 ml. to about 6 litres, and individual cysts from secretors belonging to group A have frequently been found to contain several grammes of the A-substance, which can be obtained pure by methods similar to those elaborated for the isolation of the A-substance from gastric mucin of the pig. The activity of the native cyst fluid was compared with that of known specimens of salivas on a dry weight basis by Morgan and van Heyningen, who showed that cyst fluids belonging to group A may contain up to one hundred times as much specific substance per ml. as is contained in the same quantity of a good specimen of A-saliva.

The A-substance present in the pseudo-mucinous ovarian cysts of secretors belonging to group A has been isolated by King & Morgan (1944) and its properties have been examined. The electrophoretically homogeneous substance contains 44–45 % C, 6·7 % H, 6·0 % N, 10 % CH_3CO and is weakly dextro-rotatory $[\alpha]_{5461} + 11°$. Acid hydrolysis gives rise to about 50 % of reducing sugars, 25 % hexosamine, 4·4 % of α-amino N (Van Slyke) and 2·5 % α-amino-acid N. The A-substance behaves with dilute alkali exactly as had been described for the A-substance from gastric mucin of the pig.

The active cyst fluids from secretors belonging to groups B and O offer a convenient source for the isolation of the blood-group substances. Although this has not yet been satisfactorily accomplished, preliminary experiments have shown that there is a good chance of obtaining from cyst fluids B- and O-substances in useful quantities. The recently discovered Rhesus (Rh) factors do not appear to be present in significant quantities as water-soluble components of cyst fluids obtained from secretors or non-secretors belonging to the blood groups ARh, BRh and ORh. Similarly the M and N factors, as well as the Rh factors, appear to reside largely in the erythrocytes, and are not present in the tissue fluids and secretions in a water-soluble form (Kosjakov & Tribulev, 1940; Wiener & Forer, 1941; Levine & Katzin, 1941; Boorman & Dodd, 1943).

An Artificial Blood-Group A-Agglutinogen

The results of investigations so far completed indicate that the substance present in commercial pepsin, peptone, hog gastric mucin, human saliva and pseudo-mucinous ovarian cyst fluid which possesses the serological characteristics of

the specific blood-group A-substance, is a polysaccharide-amino-acid complex. The undegraded substance is not antigenic by the ordinary tests, but possesses intense A-specific serological activity and is able to inhibit in extremely small amounts the agglutinating action of human anti-A (α) agglutinin on group A erythrocytes. The predominantly polysaccharide nature of the serologically active complex made it probable that it could be converted into an antigen showing A-specificity by means of a simple method originally used to convert certain non-antigenic specific bacterial polysaccharides and plant polysaccharides, such as agar, gum arabic and cherry gum, into antigenic complexes. These artificial antigens give rise in the rabbit to immune-body that is specific for the polysaccharide component of the antigenic complex (Morgan & Partridge, 1940, 1941; Partridge & Morgan, 1940, 1942).

The artificial complex can be made by simply mixing an aqueous solution of the A-substance with an alkaline solution of the conjugated protein component of the O somatic antigen of *Bact. shigæ* or *Bact. typhosum*. The addition of acid to the mixed solutions to yield a final *p*H of about 4·5 gives rise to the water-soluble antigenic complex. Three intravenous doses, each of 0·05 or 0·5 mg., of the artificial complex will usually give rise to potent anti-A immune-body in those rabbits that already possess a natural but low titre anti-A agglutinin. A typical immune-serum of this type will agglutinate A_1 and A_1B cells at a dilution of 1 : 25,000 to 1 : 50,000 and A_2 and A_2B cells at 1 : 2,000 to 1 : 5,000, whereas B cells are agglutinated at a dilution of 1 : 25 to 1 : 100 and O cells at less than 1 : 10. In most instances the immune-body produced is largely common α in character. Potent immune anti-A sera engendered by the artificial complex have proved to be of value in the technique of differential agglutination, and, apart from their use as blood-grouping sera, are useful reagents for the detection of weakly reacting erythrocytes belonging to sub-groups of the A-agglutinogen. In the light of these results there seems to be no reason why the water-soluble non-antigenic B- and O-specific substances present in human tissue fluids and secretions should not also be converted in B- and O-agglutinogens.

Increasing knowledge of the chemical and immunological properties of the specific group substances of erythrocytes, tissue cells, and fluids will provide a sounder basis for carrying out procedures such, for example, as the removal of unwanted isoagglutinins in human serum and plasma, the neutralisation *in vivo* by group-specific haptens of maternal isoagglutinins that give rise to fœtal death, the selection of erythrocytes that will survive longest after transfusion, and the choice of suitable cells for tissue transplantation. The success of these procedures depends upon the correct application of an exact and detailed knowledge of specific chemical and immunological relationships. An almost inexhaustible field of biochemical investigation on group antigens and specific blood-group substances awaits exploration.

REFERENCES

[*Full references to the earlier work on the specific blood-group substances are contained in Lattes, L.* (1932) "*Individuality of the blood*," *Oxford*; *Landsteiner, K.* (1936) "*The specificity of serological reactions*," *Baltimore*; *and Wiener, A. S.* (1943) "*Blood groups and transfusion*," *Baltimore*.]

Boorman, K. E. & Dodd, B. E. (1943) *J. Path. Bact.* **55,** 329
Consden, R., Gordon, A. H. & Martin, A. J. P. (1944) *Biochem. J.* (in press)
Freudenburg, K., Walsh, H. & Molter, H. (1942) *Naturwissenschaften*, **30,** 87
Fuller, A. T. (1938) *Brit. J. exp. Path.* **19,** 130
Goebel, W. F. (1938) *J. exp. Med.* **68,** 221
Hallauer, C. (1934) *Z. ImmunForsch.* **83,** 114
King, H. K. & Morgan, W. T. J. (1944) *Biochem. J.* **38,** x
Kosjakov, P. N. & Tribulev, G. P. (1940) *J. Immunol.* **37,** 297
Landsteiner, K. & Harte, R. A. (1940) *J. exp. Med.* **71,** 551
Landsteiner, K. & Harte, R. A. (1941) *J. biol. Chem.* **140,** 673
Landsteiner, K. & van der Scheer, J. (1925) *J. exp. Med.* **42,** 123
Levine, P. & Katzin, E. M. (1941) *Proc. Soc. exp. Biol., N.Y.* **48,** 126
Meyer, K. Smyth, E. & Palmer, J. (1937) *J. biol. Chem.* **119,** 73
Morgan, W. T. J. (1936) *Biochem. J.* **30,** 909
Morgan, W. T. J. (1937) *Biochem. J.* **31,** 2003
Morgan, W. T. J. (1941) *Chem. Ind.* **60,** 722
Morgan, W. T. J. (1943) *Brit. J. exp. Path.* **24,** 41
Morgan, W. T. J. & Elson, L. A. (1934) *Biochem. J.* **28,** 988
Morgan, W. T. J. & van Heyningen, R. (1944) *Brit. J. exp. Path.* **25,** 5
Morgan, W. T. J. & King, H. K. (1943) *Biochem. J.* **37,** 640
Morgan, W. T. J. & Partridge, S. M. (1939) *Nature*, **143,** 1025
Morgan, W. T. J. & Partridge, S. M. (1940) *Biochem. J.* **34,** 169
Morgan, W. T. J. & Partridge, S. M. (1941) *Biochem. J.* **35,** 1140
Morgan, W. T. J. & Partridge, S. M. (1942) *Brit. J. exp. Path.* **23,** 751
Partridge, S. M. & Morgan, W. T. J. (1940) *Brit. J. exp. Path*, **21,** 180
Partridge, S. M. & Morgan, W. T. J. (1942) *Brit. J. exp. Path.* **23,** 84
Schiff, F. (1930) *Zbl. Bakt.* I Abt. Ref. **98,** 94
Schiff, F. & Adelsberger, L. (1924) *Z. ImmunForsch.* **40,** 335
Stacey, M. (1943) *Chem. Ind.* **62,** 110
Stepanov, A. V., Kusin, A., Makajeva, Z. & Kosjakov, P. (1940) *Biochimia*, **5,** 547
Wiener, A. S. & Forer, S. (1941) *Proc. Soc. exp. Biol.*, N.Y. **47,** 215
Witebsky, E. & Klendshoj, N. C. (1940) *J. exp. Med.* **72,** 663
Witebsky, E. & Klendshoj, N. C. (1941) *J. exp. Med.* **73,** 655
Yosida, K. (1928) *Z. ges. exp. Med.* **63,** 331

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RECENT WORK ON ANÆMIAS

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During the war steady extensions have been made in our knowledge of the anæmias. There has been, perhaps, only one dramatic advance and that is the elucidation of the ætiology of erythroblastosis fœtalis.

Hypochromic Anæmias

Studies have recently been made in Britain by Davidson, Donaldson, Dyar, Lindsay & McSorley (1942, 1943, 1944), Wills, Mackay, Bingham & Dobbs (1942) and others on the wartime incidence of iron-deficiency anæmia. There appears to be a definite increase of anæmia, particularly in older children, and this has been related to the wartime diet. The importance of first-class proteins in the treatment of iron-deficiency anæmia has been rather neglected for some years except for the writings of Bethell (1936), but the work of Hahn & Whipple (1939) on dogs made anæmic by numerous bleedings at short intervals showed that, even in the presence of a large excess of iron, a low protein intake prevented the production of an adequate amount of globin and therefore of hæmoglobin. A case of severe microcytic hypochromic anæmia of long duration recently treated by the present writer gave a similar result. This patient showed only slight hæmoglobin production on intensive iron therapy, with or without vitamins and chlorophyll, but the institution of a very high protein diet caused a reticulocyte crisis and led to rapid recovery.

Much work has also been carried out over the last 10 years on the mechanism of absorption of iron, but space does not permit reference to more than a few of the papers on this subject. Whipple and his colleagues at Rochester, N.Y., have gained interesting information by the administration of radioactive "tagged" iron. Most of the experiments have been carried out on dogs, but clinical experiments in man where possible have given similar results. Thus Hahn, Bale, Ross, Balfour & Whipple (1943) found that the normal non-anæmic animal absorbs little iron, but that chronic anæmia due to blood-loss increases the absorption of iron by 5–15 times. Acute anæmia by rapid blood-loss does not at once increase the rate of iron-absorption: the iron stores of the body are first depleted and only after several days does iron-absorption increase. Gastric, duodenal and jejunal experimental pouches all show active iron-absorption.

To the number of substances which can hinder the absorption of iron from the bowel, McCance, Edgecombe & Widdowson (1943) have recently added phytic acid, which is present in whole cereals, *e.g.* brown bread. This substance causes the formation of insoluble iron phytate which passes through the bowel unchanged.

Pernicious Anæmia

The work of Fox & Castle (1942) on ætiology is a real advance and helps to correlate the pathological findings in the stomach in pernicious anæmia with the site of production of the intrinsic factor. These workers used desiccated preparations from various parts of the human stomach in the treatment of this condition and showed that the intrinsic factor was produced in the human fundus and cardiac region, and not, as in the pig, in the pyloric region of the stomach. This explains many points which were previously obscure—*e.g.* why no intrinsic factor is present in duodenal secretion, and why pernicious anæmia is very rare after partial gastrectomy.

With regard to treatment, it is being generally recognised that although the typical cases—the vast majority—of pernicious anæmia in Britain do well on highly purified liver extracts, nevertheless a definite proportion of cases met with do not do well unless one of the more crude liver extracts is used. The proteolysed liver extract described by Davis, Davidson, Riding & Shaw (1943), and now commercially available, is of particular value in some of these resistant cases.

Dyke, Della Vida & Delikat (1942) have observed the tendency of patients with pernicious anæmia in wartime to relapse in the Spring. They concluded that this was due to lack of vitamin C, as they found that these patients could be maintained on the same dosage of liver extract if a supplement of vitamin C was given at the relevant season.

Anæmias of Pregnancy

Many papers have been published on this subject in the past few years in Britain and also in India and Africa. Elliot (1944) has recently proposed a classification of these anæmias in the light of his own and other findings. While no great advances in knowledge have been made, the following generalisations may be listed. The most common type of anæmia during pregnancy in any country would appear to be the hypochromic type, largely caused by a deficiency of intake of foodstuffs rich in iron. The macrocytic type appears to be more common in tropical than in non-tropical countries, but does occur in Britain, as the 80–100 cases recorded in the last two years show—*e.g.* Fullerton (1943), Miller & Studdert (1942). In most of these cases there has also been evidence of iron deficiency. The cause of the macrocytic anæmias is complex. In some cases there is a temporary failure of production of intrinsic factor in the stomach, as shown by Strauss & Castle (1933). In others a failure of intestinal absorption, or more frequently a dietary deficiency of extrinsic factor aggravated by vomiting or anorexia is present. Bethell, Gardiner & MacKinnon (1939) attribute some of the milder forms to low-serum proteins due to a protein-deficient diet and Chatterjee (1940) has found the blood cholesterol to be very low and reports benefit from intramuscular injections of cholesterol in olive oil. In some cases it appears that the macrocytosis is a "pseudomacrocytosis" and that the anæmia is due to an acute hæmolytic process.

In most cases, treatment with parenteral liver extract, particularly the less purified forms, rapidly brings about considerable improvement and the patient satisfactorily comes to term. Occasionally some cases become hypoplastic and need repeated transfusion. Iron also should always be prescribed.

Hæmolytic Anæmias

It is now generally believed that only a very small percentage of cases of acquired acholuric jaundice are aberrant forms of the familial type. From acquired acholuric jaundice Dameshek & Schwartz (1940) and Singer & Dameshek (1941) have differentiated a group of cases under the heading "symptomatic hæmolytic anæmia," where a cause or at least a predisposing cause, such as carcinomatosis, Hodgkin's disease, etc., is present. This subject has been reviewed at length by Davis (1943), who has suggested a provisional classification of these anæmias. The term "acquired acholuric jaundice" is now usually replaced by the names subacute or chronic idiopathic hæmolytic anæmia. Most of these cases show macrocytosis, but erythrocyte fragility and microspherocytosis are variable.

Cases continue to be recorded of acute hæmolytic anæmia following various sulphonamides, but these are extremely few in relation to the number of patients treated. The prognosis is good.

The elucidation of hæmolytic disease of the newborn (erythroblastosis fœtalis) has been the most outstanding hæmatological advance of recent years. The immunisation of an Rh-negative mother by an Rh-positive fœtus, with later destruction of the fœtal erythrocytes and damage to fœtal tissues by the maternal anti-Rh antibodies so formed, is the most important cause of this disease. It must, however, be remembered that, extremely rarely, the ABO blood groups and other factors may be of ætiological significance. This subject is reviewed in more detail elsewhere,* so will not be further considered here.

Aplastic and Hypoplastic Anæmias

There is little doubt that these types of anæmia are becoming more prevalent. They constitute the most important members of the group of diverse conditions named "refractory anæmia" by Bomford & Rhoads (1941). Davidson, Davis & Innes (1943) have recently discussed a number of cases.

Many cases of aplastic or hypoplastic anæmia occur as the result of proven exposure to toxic substances, but the idiopathic type is becoming increasingly common. The treatment consists of two chief parts; first, the erythrocyte count is maintained at an adequate level by blood transfusions, which are repeated as often as necessary until the hypoplastic bone-marrow resumes its full hæmopoietic function. In this connection it is necessary to stress the importance of accurate cross-typing by the tube or other delicate method in order to detect the presence of any atypical antibodies produced in the patient's serum by the repeated transfusions. Secondly, it is usual to ensure the presence of adequate amounts of all the hæmopoietic factors by the administration of iron, liver, vitamin C and a diet containing plenty of good protein. In a certain percentage of cases after weeks, or even several months, the bone-marrow function may return to normal. If there is no improvement within six months, Whitby & Britton (1944) recommend splenectomy and record at least five successful results.

* [*see BMB* 420]

REFERENCES

Bethell, F. H. (1936) *J. Amer. med. Ass.* **107**, 564
Bethell, F. H., Gardiner, S. H. & MacKinnon, F. (1939) *Ann. intern. Med.* **13**, 91
Bomford, R. R. & Rhoads, C. P. (1941) *Quart. J. Med.* **10**, 175
Chatterjee, H. N. (1940) *Lancet*, **1**, 14
Dameshek, W. & Schwartz, S. O. (1940) *Medicine*, **19**, 231
[1] Davidson, L. S. P., Davis, L. J. & Innes, J. (1943) *Edinb. med. J.* **50**, 226, 355 & 431
[2] Davidson, L. S. P. & Donaldson, G. M. M. (1944) *Brit. med. J.* **1**, 76
[3] Davidson, L. S. P., Donaldson, G. M. M., Dyar, M. J., Lindsay, S. T. & McSorley, J. G. (1942) *Brit. med. J.* **2**, 505
[4] Davidson, L. S. P., Donaldson, G. M. M., Lindsay, S. T. & McSorley, J. G. (1943) *Brit. med. J.* **2**, 95
[5] Davis, L. J. (1943) *Edinb. med. J.* **50**, 589
[6] Davis, L. J., Davidson, L. S. P., Riding, D. & Shaw, G. E. (1943) *Brit. med. J.* **1**, 655
Dyke, S. C., Della Vida, B. L. & Delikat, E. (1942) *Lancet*, **2**, 278
[7] Elliott, G. A. (1944) *J. Obstet. Gynæc.* **51**, 198
Fox, H. J. & Castle, W. B. (1942) *Amer. J. med. Sci.* **203**, 18
Fullerton, H. W. (1943) *Brit. med. J.* **1**, 158
Hahn, P. F. & Whipple, G. H. (1939) *J. exp. Med.* **69**, 315
Hahn, P. F., Bale, W. F., Ross, J. F., Balfour, W. M. & Whipple, G. H. (1943) *J. exp. Med.* **78**, 169
McCance, R. A., Edgecombe, C. N. & Widdowson, E. M. (1943) *Lancet*, **2**, 126
[8] Mackay, H. M. M., Wills, L., Dobbs, R. H., Bingham, K., Findlay, L., Sinclair, H. M., Davidson, L. S. P., Yudkin, J. & Marshall, T. S. (1942) *Proc. roy. Soc. Med.* **36**, 69
Miller, H. G. & Studdert, T. C. (1942) *Lancet*, **2**, 332
Singer, K. & Dameshek, W. (1941) *Ann. intern. Med.* **15**, 544
Strauss, M. B. & Castle, W. B. (1933) *Amer. J. med. Sci.* **185**, 539
Whitby, L. E. H. & Britton, C. J. C. (1944) *Disorders of the blood*, 4th ed., London
[9] Wills, L., Mackay, H. M. M., Bingham, K. & Dobbs, R. H. (1942) *J. Hyg., Camb.* **42**, 505

[1] [*see BMB* **453, 454 & 455**]
[2] [*see BMB* **443**]
[3] [*see BMB* **439**]
[4] [*see BMB* **440**]
[5] [*see BMB* **447**]
[6] [*see BMB* **444**]
[7] [*see BMB* **456**]
[8] [*see BMB* **442**]
[9] [*see BMB* **441**]

CLINICAL HÆMOGLOBINOMETRY

HAROLD SCARBOROUGH, M.B., PH.D.

Although the estimation of hæmoglobin is one of the oldest most commonly employed of all laboratory methods in clinical medicine, there can be few that are carried out with less precision. This is not entirely due to fundamental defects of the methods themselves, but often to technical carelessness, to inexact and sometimes grossly inaccurate apparatus, and to the lack of suitable agreed standards to which the results can be referred. Since the development of the Gowers hæmoglobinometer in 1878, modifications of this instrument have been in constant use and the results have hitherto been considered satisfactory for clinical purposes. In recent years, however, the determination of hæmoglobin has been used as an index of shock and in the assessment of nutrition. To satisfy these demands and those of a higher standard of hæmatological investigation, a greater degree of accuracy than was hitherto considered necessary has been required. This has led to an increased interest in the principles upon which the methods are based, upon the nature of the technical procedures involved, and upon the standards of normality to be adopted (*Medical Research Council*, 1943).

A method which is to prove generally useful in clinical medicine must combine accuracy with speed and simplicity. Methods based upon the iron content of blood or upon its oxygen capacity as well as those involving the use of complicated and expensive instruments must, therefore, be reserved for research purposes and for the standardization of apparatus. With the advent, in recent years, of the cheap photoelectric cell, however, certain types of photoelectric colorimeter such as those described by King (1942) and by Bell & Guthmann (1943) are likely to be more generally used. The benzidine method of Wu may have a special sphere of usefulness in that it is said to measure all forms of hæmoglobin in as small a quantity of blood as 0·001 cm.³ with an accuracy of ± 4 % (Bing & Baker, 1931 ; Bing, 1932). From 2–12 % of the circulating hæmoglobin in apparently healthy persons may be present in an " inactive " form, probably as methæmoglobin (Ammundsen & Trier, 1939; Ammundsen, 1941), and as much as 5 % may be combined as carboxyhæmoglobin in the blood of heavy smokers. Methæmoglobin and sulphæmoglobin are frequently found in the blood of patients to whom sulphonamide drugs have been administered. A suitable method should therefore include all these forms of hæmoglobin and should be applicable to small quantities of blood obtainable without venipuncture. By reason of the fact that the blood of infants contains a form of hæmoglobin whose oxygen dissociation curve differs from that of normal hæmoglobin (McCarthy & Popják, 1943) and which is " alkali-resistant " (Brinkman & Jonxis, 1935), the determination of hæmoglobin concentration in infants may present special difficulties.

A number of methods is available which fulfil many of these requirements. The basis of all of them is the colour of oxyhæmoglobin or of one of its derivatives and, accordingly, they all depend upon the Beer-Lambert law which implies that, if the thickness of a layer of a coloured solution is maintained constant, then the concentration is proportional to the colour (or the extinction coefficient). Before this law can be used as the basis of hæmoglobinometry, however, a standard is required with which the colour of the unknown solution may be compared. The ideal standard would be derived from pure crystalline human hæmoglobin but, as this has so far not been available, an indirect method must be used instead. This is a grave disadvantage and gives rise to a number of difficulties which cannot be discussed here. It may be said, however, that no completely satisfactory standard has yet been devised.

Colour Standards

i. *Oxyhæmoglobin :* The first hæmoglobinometer devised by Gowers (Gowers, 1878) employed a dilute solution of blood compared against a standard solution of picrocarmine. Because of the difficulty of the visual comparison of these colours, this method has been abandoned. It has been shown that the colours of solutions of oxyhæmoglobin are proportional to their concentrations over a range of dilution from 1 in 1 to 1 in 5,000 (Newcomer, 1919 ; Holiday, Kerridge & Smith, 1935), and recently such solutions have been used with satisfactory results in photoelectric colorimeters such as those described by Holiday, Kerridge & Smith (1935) and by Bell & Guthmann (1943). These methods, however, do not include derivatives other than oxyhæmoglobin and may give fallacious results if the concentration of other hæm pigments is high.

ii. *Cyanmethæmoglobin* (Stadie, 1920) : This method has been extensively studied by Drabkin & Austin (1935) and adopted by Evelyn & Malloy (1938) for photoelectric use. It includes methæmoglobin and is simple, easy and accurate. It is described by King, Gilchrist & Delory (1944) as " the method of choice for research purposes " but is unsuitable for clinical use by reason of the toxic reagent required—potassium cyanide.

iii. *Pyridine-hæmochromogen* (Rimington, 1942) : This method possesses the great advantage that the standard is pure crystalline hæmin which is easy to prepare (Gattermann, 1934) and is available commercially.* It is, however, regarded as unsuitable by King, Gilchrist & Delory (1944) because the rate of colour development is not identical in the test prepared from blood and in the standard. All hæm pigments are included and the method is said to be rapid and precise when special apparatus is employed (Rimington, 1942). A satisfactory degree of accuracy for clinical purposes may, however, be achieved very simply by using a hand spectroscope as originally recommended by Roets (1940). The unpleasant smell of the pyridine will, however, be regarded by many as an insuperable objection.

iv. *Acid hæmatin :* Originally described by Sahli (1889), this method has become very popular because of its convenience. A large number of different instruments has been devised for making the colour comparison and there are almost as many artificial standards, many of which are unsatisfactory and all of which require independent standardisation. The method, though rapid and simple, is erratic and cannot be regarded as satisfactory. The acid hæmatin is not in true solution (Barcroft, 1928) and turbidity or actual precipitation may occur. The maximum colour is not developed for over 40 minutes (Newcomer, 1919) and the velocity of the colour reaction is not constant. These difficulties may be reduced by heating the solution (Ashford, 1943). Protein, lipoid, bilirubin and other constituents of plasma affect the result (Wu, 1922). Furthermore the colours are not easily matched and the personal error may be as high as ± 10 % (Heilmeyer & von Mutius, 1938). Some of the difficulties of this method have been dealt with recently by Alstead (1940). The presence of carboxy- and methæmoglobin in the blood exerts a pronounced effect on the colour development. If arrangements for an acid hæmatin hæmoglobinometer to be standardised by the British Standards Institute should mature (*Medical Research Council*, 1943) some of the present anomalies may be removed.

v. *Alkaline hæmatin* (Wu, 1922): Recent work by Clegg & King (1942) shows this method to have considerable promise. It includes all hæm compounds (including sulphæmoglobin) and is not significantly influenced by constituents of the plasma since the strong alkali has a solvent action on protein and lipoid. The alkaline hæmatin is in true solution. The colour, which is fully developed within 5 minutes in a boiling water bath, however, is not so deep as that of acid hæmatin (Ashford, 1943) and a colorimeter or photometer is desirable for matching against a standard prepared from crystalline hæmin. A dilution hæmoglobinometer of the Sahli type may, however, be adapted by substituting for the standard a tube of alkaline hæmatin (Clegg & King, 1942). In a recent investigation, King, Gilchrist & Delory (1944) found that on 20 normal bloods the standard deviations from iron analyses and oxygen capacity determinations respectively were—alkaline hæmatin 2·33 and 2·26 %; cyanmethæmoglobin 1·25 and 2·18 %; carboxyhæmoglobin (photometric) 1·70 and 2·49 %; carboxyhæmoglobin (Haldane dilution tube) 3·02 %.

vi. *Carboxyhæmoglobin* (Haldane, 1901 ; Palmer, 1918): This method is very widely used in Britain. Visual colorimetry is easier with this colour than with any other hæm

* [It may be obtained from British Drug Houses Ltd.]

pigment (*Medical Research Council*, 1943). Methæmoglobin and sulphæmoglobin are not measured, but the former may be included by preliminary reduction with sodium hydrosulphite. One of the chief causes of the unreliability of values for hæmoglobin concentration lies in the survival of the dilution colorimeter which has long since disappeared from all other biochemical estimations. Dilution colorimetry lends itself to a high degree of personal error, and in the interests of speed, convenience and accuracy, should be replaced by a constant dilution method. However, in careful hands it is probably true that the ultimate limit of visual colour discrimination ($\pm 2\%$) can be reached with a Haldane-Gowers type of hæmoglobinometer (Donaldson, Harding & Wright, 1943). The standard in this type of instrument is a dilute solution of blood, with an oxygen capacity before dilution of 18·5 volumes per cent. by the Haldane method (1899) thoroughly gassed with CO (Donaldson, Harding & Wright, 1943). This standard is remarkably permanent, having been kept unimpaired for 4–5 years (Donaldson, Harding & Wright, 1943) or even for as long as 30–40 years (Donaldson, Harding & Wright, 1943; Plesch, 1941).

In order to obviate the difficulty of obtaining "standard" blood, the Haldane standard has now been defined in terms of colour on the Commission internationale de l'Eclairage (C.I.E.) trichromatic scale (1931), and this has resulted in the British Standards Institution (B.S.I.) specification B.S. 1079: 1942.* In addition, the forms of pipettes, dilution tube and colour tube have been accurately defined and may be standardized by the British National Physical Laboratory. The hæmoglobin equivalent of the B.S.I. Haldane colour standard has now been evaluated (Macfarlane & O'Brien, 1944; King, Gilchrist & Matheson, 1944) as equivalent to an oxygen capacity of $19{\cdot}7 \pm 0{\cdot}2$ ml. oxygen per 100 ml. blood and to an iron content of $49 \pm 0{\cdot}8$ mg. iron per 100 ml. The definition of the standard in terms of colour, the standardization of all apparatus and the observations set forth in *Recommendations of standard methods for clinical determination of hæmoglobin* (*Medical Research Council*, 1943) should ensure a higher degree of accuracy in the determination of hæmoglobin concentration.

Photoelectric Methods

Although it has been stated that colour differences of 2% on the Haldane scale are just appreciable with a dilution colorimeter of the Haldane-Gowers type (*Medical Research Council*, 1943), many clinical workers find it impossible to achieve this degree of accuracy. The sorts of errors which can occur in clinical practice with old and unstandardized apparatus have been reported on by Hay (1943). There can be no doubt that the further development of photoelectric methods offers the most hopeful line of advance for the assessment of the actual colour of the compound chosen and so for the concentration of hæmoglobin in solution. Many of the difficulties inherent in photoelectric estimations are overcome by using the Brice (1937) balanced circuit as described by Reeve (1944). Some of these difficulties have been referred to by Peterson & Strangeways (1944). It must be remembered, however, that the presence in the sample of different hæmoglobin compounds with differing absorption spectra may interfere with the direct relationship between light absorption and hæmoglobin concentration and, secondly, that other materials such as constituents of plasma, slight turbidity of the solutions, etc., may significantly affect the result. A simple, rapid and trustworthy photoelectric method for the determination of hæmoglobin has recently been described by Reeve (1944). By utilizing the total absorption of hæmoglobin and oxyhæmoglobin in the green, this method achieves an accuracy of $\pm 2\%$ hæmoglobin. If hæmoglobin pigments be converted into cyanhæmoglobin the procedure becomes more sensitive—a change of 0·5% can be detected with average care. The presence of relatively large amounts of sulphæmoglobin, which is not convertible to cyanhæmoglobin, affects the result, but the presence of such amounts is detected during the estimation.

Finally it should be mentioned that the practice introduced by Hayem of expressing the hæmoglobin concentration as a percentage of an arbitrary normal figure has led to much confusion by reason of the fact that concentrations of hæmoglobin varying between 12·4 and 17·3 g. per 100 ml. have been accepted as equivalent to 100%. The logical way to avoid this chaotic state is to express hæmoglobin values in terms of actual concentration without being committed to any arbitrary scale of normals. This "13·8 g. of hæmoglobin per 100 ml. of blood" is an accurate way of expressing a concentration of hæmoglobin which might be recorded as 111% on one scale and 80% on another.

Note

Testing of Haldane hæmoglobinometer colour tubes and graduated tubes

The *National Physical Laboratory* is prepared to accept hæmoglobinometer colour tubes and graduated hæmoglobinometer tubes for test of their conformity with the *British Standards Specification* (B.S. 1079: 1942). Full particulars of the tests and the fees charged may be obtained through the Editor of *BMB*.

* [Readers not resident in the United Kingdom who require a copy of this specification should apply to the Editor of *BMB*.]

REFERENCES

Alstead, S. (1940) *Post Grad. med. J.* **16**, 278
Ammundsen, E. (1941) *J. biol. Chem.* **138**, 563
Ammundsen, E. & Trier, M. (1939) *Acta med. Scand.* **101**, 451
Ashford, C. A. (1943) *Brit. med. J.* **2**, 575
Barcroft, J. (1928) *The respiratory function of the blood: Vol. 2. Hæmoglobin*, Cambridge, p. 17
[1] Bell, G. H. & Guthmann, E. (1943) *J. sci. Instrum.* **20**, 145
Bing, F. C. (1932) *J. biol. Chem.* **95**, 387
Bing, F. C. & Baker, R. W. (1931) *J. biol. Chem.* **92**, 589
Brice, B. A. (1937) *Rev. sci. Instrum.* **8**, 279
Brinkman, R. & Jonxis, J. H. P. (1935) *J. Physiol.* **85**, 117
C.I.E. (1931) Commission internationale de l'Eclairage. *Compte rendu des séances*, Cambridge, p. 19
[2] Clegg, J. W. & King, E. J. (1942) *Brit. med. J.* **2**, 329
Donaldson, R., Harding, H. G. W. & Wright, G. P. (1943) *J. Path. Bact.* **55**, 205
Drabkin, D. L. & Austin, J. H. (1935) *J. biol. Chem.* **112**, 51, 67 & 89
Evelyn, K. A. & Malloy, H. T. (1938) *J. biol. Chem.* **126**, 655
Gattermann, L. (1934) *Laboratory methods of organic chemistry*, London, p. 395.
Gowers, W. R. (1878) *Practitioner*, **21**, 1
Haldane, J. S. (1899) *J. Physiol.* **25**, 295; (1901) *J. Physiol.* **26**, 497
Hay, S. (1943) *Edinb. med. J.* **50**, 626
Heilmeyer, L. & von Mutius, I. (1938) *Dtsch. Arch. klin. Med.* **182**, 164
Holiday, E. R., Kerridge, P. M. T. & Smith, F. C. (1935) *Lancet*, **2**, 661
King, E. J. (1942) *Lancet*, **1**, 511
King, E. J., Gilchrist, M. & Delory, G. E. (1944) *Lancet*, **1**, 239
King, E. J., Gilchrist, M. & Matheson, A. (1944) *Brit. med. J.* **1**, 250
McCarthy, E. F. & Popják, G. (1943) *Biochem. J.* **37**, xviii
Macfarlane, R. G. & O'Brien, J. R. P. (1944) *Brit. med. J.* **1**, 248
Medical Research Council (1943) *Brit. med. J.* **1**, 209
Newcomer, H. S. (1919) *J. biol. Chem.* **37**, 465
Palmer, W. W. (1918) *J. biol. Chem.* **33**, 119
Peterson, J. M. & Strangeways, D. H. (1944) *Brit. med. J.* **1**, 43
Plesch, J. (1941) *Brit. med. J.* **2**, 859
Reeve, E. B. (1944) *J. Path. Bact.* **56**, 95
Rimington, C. (1942) *Brit. med. J.* **1**, 177
Roets, G. C. S. (1940) *Onderstepoort J. vet. Sci.* **14**, 451
Sahli, H. (1889) *Lehrbuch der klinischen Untersuchungs-Methoden*, Leipzig
Stadie, W. C. (1920) *J. biol. Chem.* **41**, 237
Wu, H. (1922) *J. Biochem., Tokio*, **2**, 173

[1] [see *BMB* 470]
[2] [see *BMB* 469]

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CIRCULATORY DYNAMICS OF HÆMORRHAGE

E. P. SHARPEY-SCHAFER, M.R.C.P.

It is now generally agreed that hæmorrhage is the major factor requiring treatment in casualties with low blood-pressure. Studies of circulatory dynamics after hæmorrhage in man, as opposed to the experimental animal, are of special interest, for it appears that there may be some species-difference, for example, in the time taken for the restoration of

blood volume. In man, dilution of hæmoglobin and restoration of blood volume after bleeding is slow (Ebert, Stead & Gibson, 1941; Wallace & Sharpey-Schafer, 1941). Further, new methods are becoming available for study of circulatory dynamics which are particularly applicable to unanæsthetised man.

Methods

Blood-volume determination continues to present a difficult problem. Using the Evans-blue method, Morris (1944) points out that hæmolysis in the plasma samples interferes with the analysis, as hæmoglobin is converted by the extraction process into a pigment which has a significant light absorption in the same spectral region as Evans-blue. He describes an optical method for correction of this error. Dyson, Plaut & Vaughan (1944) made observations on the changes in erythrocytes, hæmoglobin, mean corpuscular volume, serum protein and plasma volume in a two-hour period following the removal of 540 cm.³ of blood in 8 normal subjects and following transfusion of concentrated erythrocytes in 8 anæmic subjects. Plasma volume was estimated by the Harington, Pochin & Squire (1940) modification of the Evans-blue method of Gibson & Evans (1937). They found a considerable variation in the response of different individuals. In one instance the plasma volume after bleeding was that calculated; in the others dilution or overdilution were noted. Plasma proteins showed either no change or a fall. The data after concentrated corpuscle transfusions suggested that there might either be a small increase in plasma volume (442 cm.³ to 262 cm.³) above that calculated or else a small decrease (241 cm.³ to 253 cm.³). The authors consider that in estimating the amount of fluid that has passed from the tissues into the circulation, the amount absorbed by the erythrocytes is significant and must be taken into account.

McMichael, Sharpey-Schafer, Mollison & Vaughan (1943) measured the blood volume in severe anæmia by a concentrated corpuscle-hæmoglobin method (Hill, 1941) according to the formula: Blood volume (cm.³) $= \frac{V(Hb_v - Hb_2)}{Hb_2 - Hb_1}$ where V cm.³ = the volume of concentrated corpuscles injected (usually about 500 cm.³), Hb_v = hæmoglobin percentage of injected corpuscles, Hb_1 = hæmoglobin percentage of patient before transfusion, and Hb_2 = hæmoglobin percentage after transfusion. Hæmoglobin was estimated by a photoelectric method. In 7 cases, simultaneous estimations were made by a concentrated-corpuscle-differential agglutination method, and in 3 by the Evans-blue dye method. Results agreed within ½ litre. The concentrated-corpuscle-hæmoglobin method can be used only in severe anæmia.

States of low blood-pressure can be analysed on the principle: mean blood-pressure = cardiac output per unit time × total peripheral resistance. It is now possible to make accurate serial estimations of cardiac output in man by the method of cardiac catheterisation. First used by Forssmann (1929) and later by de Carvalho & Moniz (1933) and Ameuille, Ronneaux, Hinault, Desgrez & Lemoine (1936), this technique was developed by Cournand & Ranges (1941) for measuring cardiac output by a direct Fick method. The procedure is simple and safe. It consists, essentially, of passing a radio-opaque gum-elastic ureteric catheter along the veins of the arm into the right auricle, where the position of the tip is checked by *x*-rays. The pressure in the right auricle can be recorded by a citrate or saline manometer, and the oxygen unsaturation of mixed venous blood can be measured by withdrawing suitable samples under oil. Details of the procedure are given in the papers of Cournand and his colleagues and by McMichael & Sharpey-Schafer (1944). Over 500 cardiac catheterisations have now been performed without complication.

Factors Affecting Cardiac Output in Normal Subjects

Results obtained in normal subjects (McMichael & Sharpey-Schafer, 1944) were those to be expected from the well-known work of Starling and of Wiggers (1927). Reduction of the right auricular pressure by bleeding caused a fall in cardiac output, and raising the right auricular pressure by rapid transfusion caused a rise. Accelerating the heart by intravenous injection of atropine usually caused an increased cardiac output. However, there was also a fall in right auricular pressure, and this was sometimes sufficient to counteract the usual increase of output on acceleration. Intravenous adrenaline in doses that did not raise the blood-pressure or accelerate the heart (under 10 μg. per minute) increased cardiac output, presumably by a direct action on the heart itself. Cardiac output in the supine posture showed an average increase of 33% over that in the erect subject.

Immediate Effects of Hæmorrhage

Apart from its importance in the "shock" problem, a sudden fall of blood-pressure after bleeding has been of particular interest to transfusion units. Poles & Boycott (1942) found that 3·8% of young factory workers fainted after removal of 440 cm.³ of blood, and 8·5% fainted after removal of 540 cm.³ They thought that the incidence of fainting was raised by bleeding tired and hungry subjects. Glucose (40 g.) given prophylactically was without effect, but 1 litre of saline before bleeding seemed to be beneficial in men from hot workshops. In a report to the *Medical Research Council* by a sub-committee of the Blood Transfusion Research Committee (*Medical Research Council*, 1944) an analysis of data was given from 362 blood-donors who had fainted. The results showed that the incidence of fainting was lower in men than in women, but there was no evidence that age, length of wait at the centre, difficulties in bleeding or menstruation had any effect on the incidence of fainting. The most striking finding was that a high proportion of fainters gave a history of fainting either at a previous donation or on some other occasion. Loutit, Mollison & van der Walt (1942) were able to confirm previous work in showing that venesection lowered and transfusion raised the peripheral venous pressure. They found little change in blood-pressure and heart-rate in 20 donors bled of 430 cm.³ However, with larger venesections (up to 1100 cm.³) the incidence of acute fall of blood-pressure and bradycardia ("faint") was much higher (Wallace & Sharpey-Schafer, 1941). By placing venous tourniquets on the thighs for 20 minutes at diastolic pressure, or better at 10 to 15 mm. Hg. below systolic pressure, and then bleeding 500 cm.³ or more, a very high incidence of fainting can be produced in normal volunteers. This procedure is probably equivalent to a venesection of about 1400 cm.³, and has the advantage that release of the tourniquets will rapidly restore blood-pressure and consciousness if the faint is severe. Using such large venesections in normal male volunteers in the supine position Barcroft, Edholm, McMichael & Sharpey-Schafer (1944) studied the physiology of post-hæmorrhagic fainting by observing cardiac output (cardiac catheterisation) and forearm flow (Lewis and Grant plethysmograph). During the bleeding, blood-pressure was maintained but right auricular pressure and cardiac output fell steadily, indicating that while blood-pressure was kept at a normal level by increasing total peripheral resistance (probably vasoconstriction in skin and splanchnic areas), yet there was no corresponding increase of venous tone to maintain right auricular pressure. The acute fall of blood-pressure and bradycardia (fainting) were not due to any further fall in cardiac output but to a sudden great decrease in total peripheral resistance. During the faint, forearm flow was approximately doubled when the blood-pressure was halved, indicating great arteriolar vasodilatation in forearm muscles, since the skin is pale during the faint. If the rest of the skeletal muscle behaves like the forearm, then vasodilatation in muscle may explain the fall of blood-pressure in post-hæmorrhagic fainting. Barcroft & Edholm (1944) have further shown that this vasodilatation is mediated by vasomotor nerves. On the afferent side it is possible that the fall of pressure in the right auricle may be a trigger mechanism. Retransfusion immediately restored forearm flow and blood-pressure to normal. In two cases intramuscular methedrine (30 mg. N-methyl amphetamine) restored blood-pressure by increasing total peripheral resistance; this substance can also be given intravenously.

Late Effects of Hæmorrhage

Data are still scanty on the phase of "shock" after hæmorrhage. This is the phase of low blood-pressure, with normal or increased heart-rate, which is commonly seen in the wards after spontaneous bleeding and responds well to transfusion. The evidence of Cournand and his colleagues indicates that low right auricular pressure and low cardiac output are the main factors causing low blood-pressure. In some of their cases total peripheral resistance was increased, and in others cardiac output was not as low as might be expected. Oxygen consumption might be slightly reduced but arterial oxygen-saturation was normal; there seems little

purpose, therefore, in giving oxygen. At the present time Cournand and his colleagues think that pressor substances should not be used, as vaso-constriction is probably already optimal in this phase, and that hyperventilation should not be depressed with morphine. It seems probable that the "faint" may supervene on the "shock" phase if any procedure, such as a change to the sitting or upright posture, further reduces right auricular pressure.

In the course of spontaneous recovery from a large hæmorrhage, the circulation often enters a third phase which may be termed the "hyperkinetic syndrome" (Harrison, 1939). Estimation of blood volume in post-hæmorrhagic and chronic anæmia with normal systolic blood-pressure showed surprisingly low figures, often as low as 2 litres (McMichael *et al.*, 1943). Further work (Sharpey-Schafer, 1944) showed that the normal or slightly raised resting oxygen supply in severe anæmia was maintained by three main factors (i) increased cardiac output, (ii) increased utilisation of available arterial oxygen, (iii) diminished blood volume resulting in greater concentration of total circulating hæmoglobin. Increased cardiac output could be due to a moderate increase of heart-rate combined with a normal or raised venous pressure. In the most severe cases the right auricular pressure was elevated, sometimes to high levels. This rise of venous pressure or "heart failure" was thought to be the result of an adjusting mechanism, perhaps increased venous tone, which served to maintain the necessarily high cardiac output.

It is clear that a circulatory phase in which the blood volume is low, and the venous pressure and cardiac output are increased, is likely to give trouble when further overloaded by transfusion. Drummond (1943) has recorded some excellent clinical examples; three of his five cases had hæmoglobin levels of 25 % or less and all appear to have developed pulmonary œdema, in one case after as little as 110 cm.[3] of blood. The three patients who died had well-marked pulmonary œdema, and there was great ballooning of the right auricle in a patient who had received about 2½ litres. When more exact studies are made it is found that, in severe anæmia, raising the right auricular pressure further by a transfusion may have precisely the opposite effect to the same procedure in a normal subject. Instead of increasing cardiac output, as in the normal, it causes a decrease. The situation seems in every way comparable to Starling's overloaded heart-lung preparation. Patterson & Starling (1914) showed that increasing the venous pressure caused a corresponding increase in cardiac output, until a point was reached after which further increase of venous pressure caused a fall in output.

At the present time the evidence indicates that, following hæmorrhage, venous pressure changes are of considerable importance both in the process of spontaneous recovery and in assessing the effects of transfusion. If more accurate methods of measurement are not available, clinical observation of the neck veins by the well-known methods described by Sir Thomas Lewis may give valuable information.

REFERENCES

Ameuille, P., Ronneaux, G., Hinault, V., Desgrez, — & Lemoine, J. M. (1936) *Bull. Soc. méd. Hôp. Paris*, **52**, 729
Barcroft, H. & Edholm, O. G. (1944) [*Unpublished report to Medical Research Council*]
Barcroft, H., Edholm, O. G., McMichael, J. & Sharpey-Schafer, E. P. (1944) *Lancet*, **1**, 489
de Carvalho, L. & Moniz, E. (1933) *Acta. radiol. Stockh.* **14**, 431
Cournand, A. & Ranges, H. A. (1941) *Proc. Soc. exp. Biol. N.Y.* **46**, 462
Cournand, A., Riley, R. L., Bradley, S. E., Breed, E. S., Noble, R. P., Lauson, H. D., Gregersen, M. I. & Richards, D. W. (1943) *Surgery*, **13**, 964
Drummond, R. (1943) *Brit. med. J.* **2**, 319
Dyson, M., Plaut, G. & Vaughan, J. (1944) *Quart. J. exp. Physiol.* **32**, 255
Ebert, R. V., Stead, E. A. & Gibson, J. G. (1941) *Arch. int. Med.* **68**, 578
Forssmann, W. (1929) *Klin. Wschr.* **8**, 2085
Gibson, J. G. & Evans, W. A. (1937) *J. clin. Invest.* **16**, 301 & 317
Harington, C. R., Pochin, E. E. & Squire, J. R. (1940) *Clin. Sci.* **4**, 311
Harrison, T. R. (1939) *Failure of the circulation*, 2nd ed. London
Hill, D. K. (1941), *Lancet*, **1**, 177
[1] Loutit, J. F., Mollison, M. D. & van der Walt, E. D. (1942) *Brit. med. J.* **2**, 658
McMichael, J. & Sharpey-Schafer, E. P. (1944) *Brit. Heart J.* **6**, 33
[2] McMichael, J., Sharpey-Schafer, E. P., Mollison, P. L. & Vaughan, J. M. (1943) *Lancet*, **1**, 637
[3] Medical Research Council (1944) *Brit. med. J.* **1**, 279
[4] Morris, C. J. O. R. (1944) *J. Physiol.* **102**, 441
Patterson, S. W. & Starling, E. H. (1914) *J. Physiol.* **48**, 357
[5] Poles, F. C. & Boycott, M. (1942) *Lancet*, **2**, 531
Sharpey-Schafer, E. P. (1944) *Clin. Sci.* [*in press*]
Wallace J. & Sharpey-Schafer, E. P. (1941) *Lancet* **2**, 393
Wiggers, C. J. (1927) *J. Pharmacol.* **30**, 233

[1] [*see BMB* 477] [2] [*see BMB* 479] [3] [*see BMB* 478] [4] [*see BMB* 481] [5] [*see BMB* 476]

REVIEW OF SELECTED PAPERS

Blood Groups

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THE Rh FACTOR AND ERYTHROBLASTOSIS FŒTALIS: AN INVESTIGATION OF 50 FAMILIES

by R. R. Race, G. L. Taylor, D. F. Cappell & M. N. McFarlane, *British Medical Journal*, **2**, 289–293, 4/9/43

Workers from the *Medical Research Council* Serum Unit and from the University of St. Andrews have collaborated in the investigation of 50 families in which erythroblastosis fœtalis, better called hæmolytic disease of the newborn, has been diagnosed. The cases were collected in many parts of Great Britain and Northern Ireland, and in those reported it seemed reasonably certain that the diagnosis was correct. An attempt was made to determine the ABO and Rh blood groups of parents and surviving children and to get details of all pregnancies and their outcome. The authors point out that although the material is far from complete, much information has been gathered together and some of it may well prove of value to future workers. The details relating to the families are tabulated.

Of the 50 mothers, 6 were Rh-positive and 44 Rh-negative, and in the sera of 38 of the Rh-negatives, anti-Rh agglutinins were found. These figures agree well with those of other workers and confirm Levine's theory that iso-immunization of the mother plays an important part in the ætiology of hæmolytic disease of the newborn. Anti-Rh was not found in 6 of the Rh-negative mothers, but the fact that 6 of the 12 mothers in whose serum anti-Rh was not found were Rh-negative strongly suggests that Rh was concerned.

In one pregnancy in ten the mother is Rh-negative and the baby Rh-positive, and in one pregnancy in five the mother has an agglutinin for an ABO antigen present in her fœtus, but the disease is much rarer [1 in 200–400 pregnancies] than it would be if it occurred whenever the blood-group situation makes it possible. Other factors yet unknown must be involved.

When the mother is Rh-positive (or even Rh-negative), other erythrocyte antigens than Rh may perhaps cause the disease. The husbands of only 2 of the 6 Rh-positive mothers were grouped. One was O, Rh-negative; the other A, Rh-negative. After the investigation of the 50 families had been made, the next two husbands of Rh-positive mother cases examined were negative and positive. It appears significant that three of four husbands of Rh-positive mothers with affected children should be Rh-negative. The family in which both parents were Rh-positive produced St serum and is described at length by McCall, Race & Taylor (1944). Families in which the diagnosis is wrong will most commonly be included amongst those with Rh-positive mothers, simply because six out of seven mothers in the population are Rh-positive.

Not one Rh-negative child was found amongst the offspring

of the Rh-negative mothers; 33 healthy and 16 affected children were tested. The absence of negative children from the healthy group is highly significant and may be due to most of the fathers being homozygous (RhRh) and producing only Rh-positive children. Immunization is more likely when an Rh-negative mother has a positive fœtus every pregnancy than when some may be positive and others negative, as with a heterozygous (Rhrh) husband. Although negative children do occur in affected families, the scarcity has to be considered in estimating the chances of getting a negative (an unaffected) child in future pregnancies. In the 50 families, with one exception, no mother who had once produced an affected baby ever had a later normal surviving child.

Of the first children borne by the 44 Rh-negative mothers 38 were unaffected, five were stillbirths or miscarriages and one suffered from the disease. From the second birth onwards there was a steadily increasing ratio of affected to normal children. Four of the six Rh-positive mothers, on the other hand, had first children who were affected, two being stillbirths. The earlier onset in this group might be due, if the ABO groups were responsible, to the iso-agglutinins being already present in the maternal serum.

About a quarter of the affected children survived; three-quarters were born dead or died mostly within a week of birth. Affected children were approximately evenly distributed between the two sexes.

REFERENCE

[1] McCall, A. J., Race, R. R. & Taylor, G. L. (1944) *Lancet*, **1**, 214

[1] [*see BMB* **431**.]

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HÆMOLYTIC DISEASE OF THE NEWBORN (ERYTHROBLASTOSIS FŒTALIS): ITS TREATMENT WITH RHESUS-NEGATIVE BLOOD

by J. D. Gimson, *British Medical Journal*, **2**, 293–297, 4/9/43

The author reports in much detail a series of 19 consecutive cases of hæmolytic disease of the newborn collected during eleven months at the *Hospital for Sick Children*, Great Ormond Street, London. The cases are typical of those coming to a children's hospital which is not part of a maternity hospital, and are necessarily selected, for macerated fœtuses and stillborn infants will never be seen, and hydrops fœtalis or icterus gravis—fatal within 24 hours—will be admitted only rarely. All the mothers were Rh-negative and all the infants Rh-positive, and anti-Rh was found in the sera of all but one of the mothers. Some cases, even of icterus gravis, recover without treatment, but the mortality seems to be about 80%.

Knowledge of the connection between iso-immunization of the mother and hæmolytic disease of the newborn has suggested the treatment of the child by the transfusion of blood lacking the antigen for which the mother has made the agglutinin. Attempts to replace by transfusion the baby's blood lost by lysis have in the past not been very successful, because in most cases Rh is involved. As six donors out of seven are Rh-positive and their blood would not have been Rh-tested, Rh-positive blood would usually have been given, and this, like the baby's own, was liable to be destroyed.

Eighteen of the 19 babies, whose ages on admission varied between three days and six weeks, were transfused with Rh-negative blood free from anti-Rh. It was rarely thought necessary to transfuse unless the erythrocyte count was below 3·5 millions.

All the babies received Rh-negative blood, and some of the earlier ones were given Rh-positive blood as well; but, although the positive blood caused an initial rise in hæmoglobin and erythrocytes, hæmolysis continued, and within a few days further transfusion was needed. Rh-negative blood does not prevent hæmolysis of the child's own cells, it merely provides cells which will not be destroyed more rapidly than normal, and on which the child may live until the lytic process ends. When a mixture of Rh-positive and Rh-negative blood was given the negative cells survived normally while the positive were often destroyed within a few days. In 14 of the 18 cases the results are described as "perfect"; the other 4 regained and maintained normal blood pictures. No more than two transfusions of negative blood were needed in any case.

The author shows how encouraging are the results in this series by comparing them with those from a series of 17 consecutive cases treated in the same hospital with blood which, untested for Rh, would nearly always be Rh-positive. Six of the 17 died from the disease or from transfusion; 4 were kept alive but without improvement in the blood condition; and in only 7 were the results satisfactory, although they received much more blood—up to six transfusions in some cases—than the 18 babies treated with Rh-negative blood. Moreover, no reactions followed the giving of negative blood, but in the other series there was more often than not a rise of temperature and some constitutional disturbance with increase of jaundice and size of liver and spleen.

The transfusions were given intravenously, by drip into the internal saphenous or a cubital fossa vein. An attempt was made to maintain a constant rate of 15 to 20 cm.^{3} per hour. The infants were kept in hospital overnight after the transfusion. The volume of blood to be given was calculated in cm.^{3} from the formula

$$\frac{\%\text{ rise of Hb required}}{100} \times \text{blood volume}$$

the blood volume being approximately 88 cm.^{3} per kg. body weight calculated on expected weight for age from birthweight. The author concludes that if an infant with hæmolytic disease of the newborn lives long enough to reach hospital there is a good chance of its survival. She thinks it unlikely that the dreaded kernicterus can be avoided.

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OCCURRENCE OF THE Rh ANTIGEN IN THE POPULATION: NOTES ON 5 CASES OF ERYTHROBLASTOSIS FŒTALIS

by E. D. Hoare, *British Medical Journal*, **2**, 297–298, 4/9/43

The author, working in the Welsh National School of Medicine, immunized 10 guinea-pigs with Rhesus monkey blood as described by Landsteiner & Wiener (1941). Three of the immunized animals yielded sera which, at dilutions up to 1 : 60, gave a clear distinction between Rh-positive and Rh-negative bloods. By tests made with these sera it was found that the distribution of the Rh-positive and Rh-negative persons in South Wales agrees with that found in England and amongst the white people in the United States of America. The Welsh results were:

Group	*AB*	*A*	*B*	*O*	*Total*
Rh-positive .	27	349	90	483	949 (84·6%)
Rh-negative .	3	64	14	92	173 (15·4%)

Five cases of erythroblastosis fœtalis are reported, in all of which the father and child were Rh-positive whilst the mother was Rh-negative. Anti-Rh was found in the sera of the five mothers. In one of the sera the agglutinin seems to have been undetectable at 37° C.; it was found at room temperature in the undiluted serum and to a titre of 1 : 4 at 0° C. Four of the children were transfused with blood which was not tested for Rh: only one of them lived. The fifth child died without transfusion. These cases occurred before the need of treatment with Rh-negative blood had been realised.

REFERENCE

Landsteiner, K. & Wiener, A. S. (1941) *J. exp. Med.* **74**, 309

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THE SURVIVAL OF TRANSFUSED ERYTHROCYTES IN HÆMOLYTIC DISEASE OF THE NEWBORN

by P. L. Mollison, *Archives of Disease in Childhood*, **18**, 161–172, December 1943

This paper is a report to the *Medical Research Council* from the South-West London Blood Supply Depot, and records the first direct observations of the survival of transfused blood in the circulation of babies with hæmolytic disease of the newborn. Eighteen babies suffering from this disease were transfused with Rh-negative blood and 10 of them were given Rh-positive blood as well, either at the time in a mixture or consecutively. The survival of each type of donor's erythrocytes was followed by a very ingenious modification of the Ashby (1919) technique. An example is

given: a baby OM Rh-positive was transfused with a mixture in known proportions of OM Rh-negative and ON Rh-positive blood. After transfusion the baby's blood was agglutinated by anti-Rh serum, the unagglutinated cells being those of the first donor, and these were counted. The baby's blood was next agglutinated by anti-M serum; this time the unagglutinated cells were those of the second donor and could also be counted. Corrections have to be made for the number of cells failing to be agglutinated by their homologous serum.

The results were conclusive and fully confirmed the correctness of the theoretical expectation and of the clinical impressions. If Rh-positive blood was given in the first fortnight of life it was practically all eliminated within ten days (in 3 cases elimination was complete in three days). If the baby was older, Rh-positive blood was tolerated better. Compared with this, 90 % of the Rh-negative transfused cells were still surviving at the end of a week, and their total survival was about that of compatible transfused cells in the adult.

The author therefore considers that when an infant with hæmolytic disease of the newborn has to be transfused, Rh-negative blood of its own group should be given when serological tests have been made and it is fairly certain that destruction is due to anti-Rh agglutinins. O Rh-negative blood is recommended when no tests have been made, and O blood should be used when destruction is thought to be due to anti-A or anti-B agglutinins. When destruction is due to agglutinins other than typical anti-Rh, O blood compatible with the mother's serum is probably the safest. In every case direct matching of the donor erythrocytes against the mother's serum seems a desirable precaution before transfusion.

An interesting additional observation is recorded which may be of physiological importance. One baby expected to suffer from hæmolytic disease of the newborn was transfused at birth, while its Rh grouping was being done. The baby was Rh-negative, however, so the transfusion was stopped, 80 cm.³ having been given. Two days later the hæmoglobin was over 150 % (Haldane), and it was still 134 % 10 days after birth. During these 10 days there was not the slightest tinge of jaundice. The author considers that this calls in question the generally accepted theory that physiological jaundice results from a process designed to destroy the excessive numbers of erythrocytes needed *in utero* but unwanted in the open air.

REFERENCE

Ashby, W. (1919) *J. exp. Med.* **29**, 267

430

HÆMOLYTIC DISEASE IN THE NEWBORN: THE Rh FACTOR

by F. A. Langley & F. Stratton, *Lancet*, **1**, 145–148, 29/1/44

This is a review of the clinical, pathological and serological findings in 23 carefully investigated cases of hæmolytic disease of the newborn. Twenty-two of the mothers were Rh-negative and anti-Rh was found in the serum of 19 of them.

As a control, 41 cases of physiological jaundice were also examined. The number of Rh-negatives (5) amongst the mothers of these controls was close to that expected in a random sample of women. This suggests that when the cases are carefully diagnosed Rh is seldom found to be the cause of physiological jaundice. However, one of the five Rh-negative mothers had anti-Rh in her serum.

The authors point out the difficulties that may be met in making a diagnosis of hæmolytic disease, since important indications such as jaundice or erythroblastæmia may be absent. In their experience "the most important single clinical feature was a progressive fall in the hæmoglobin level below that normally expected." Post-mortems were performed on twelve of the babies and the findings are reviewed.

Some interesting points of technique are mentioned. First and most important, the authors do not consider high-titre human anti-A and anti-B serum adequate for the ABO grouping of babies. Immune rabbit sera were much more satisfactory in their experience.

There is a good description of a certain type of clumping familiar to Rh workers which must not be confused with true agglutination. These clumps are due to gentle handling of sedimented cells and were described as "large almost circular clumps shedding cells as they moved, which gave them a comet-like appearance."

The authors were able to demonstrate anti-Rh agglutinin in the milk of 7 out of 9 mothers who were known to have the antibody in their serum.

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RHESUS ANTIBODY IN Rh-POSITIVE MOTHER CAUSING HÆMOLYTIC DISEASE OF NEWBORN

by A. J. McCall, R. R. Race & G. L. Taylor, *Lancet*, **1**, 214–215, 12/2/44

In this paper are given the clinical details and history of the family of the Rh-positive mother from whom was obtained the antiserum St which has proved of fundamental importance in the recognition of allelomorphs of the Rh gene and resulting genotypes. Investigation of this unusual serum, from an Rh-positive mother, yet capable of agglutinating most Rh-positive cells, showed that it was similar to, but not necessarily identical with, the anti-Hr discovered by Levine and his colleagues in America. St serum reacts with 80 % of people including all Rh-negatives, all heterozygotes (Rhrh) and some of the homozygotes (RhRh). These reactions are due to the presence in the erythrocytes of antigens determined by allelomorphs of the Rh gene. The use of St with other forms of anti-Rh sera has made it possible to determine serologically the genotype of about 80 % of people. The genotype testing has been described in previous papers (Race & Taylor, 1943; Race, Taylor, Boorman & Dodd, 1943; Race, Taylor, Cappell & McFarlane, 1944).

A male infant in whom increasing jaundice had been noticed from the 2nd day was admitted to hospital on the 14th day with pallor, deep jaundice and a moderately enlarged liver. The stools and urine were deeply bile-stained. Hb 55 % (9 g. per 100 ml.), erythrocytes 2,500,000 per mm.³, colour index 1·1, mean diameter of erythrocytes 8·1μ, leucocytes 15,000 per mm.³ Anisocytosis and polychromasia well marked; 300 normoblasts per mm.³; blood group O, Rh-positive. On the 16th day the Hb was 42 %. The mother was not available for testing, so 150 ml. of Rh-negative blood was transfused. The mother proved to be Rh-positive, and her serum contained a powerful agglutinin which acted on the cells of the Rh-negative donor used to transfuse the baby and on all of 12 unselected group O bloods. Agglutination occurred at room temperature but was stronger at 37° C. No agglutinin for these cells was found in the child's serum.

On the 22nd day Hb was 68 % and on the 27th 46 %. Of 20 group O bloods examined, the mother's serum failed to agglutinate 3, either at 37° C. or at room temperature. Transfusion of 200 ml. of blood from one of the 3 St-negative donors raised the Hb to 79 %, a level which was maintained, and the child was discharged free from jaundice on the 57th day.

The mother, who suffered from a severe hypochromic anæmia aggravated by post-partum hæmorrhage, was transfused with 600 ml. of St-negative group O blood without reaction.

Family history: Mother had had no stillbirths or abortions.

1st child, male, 1939. Healthy, no history of erythroblastosis. Group ON, P−, Rh+, St+ (Rh_1rh).

2nd child, male, August 1941. Died from erythroblastosis. No investigations.

3rd child, male, January 1943. History given above. Group OMN, P−, Rh+, St+ (Rh_1rh).

Mother. Group OMN, P−, Rh+, St− (Rh_1Rh_1).

Father. Group A_1MN, P?, Rh+, St+ (Rh_1rh).

St serum does not react with Rh_1, and the father and children (all Rh_1rh) are St + because they possess the recessive gene rh. The mother (Rh_1Rh_1), not having rh, was immunized by the child's rh. St always reacts with rh both in the heterozygous Rhrh and in the Rh-negative rhrh. St also reacts with Rh_2 [and with Rh″, Rh_0 and Rh_y].

[In transfusing the baby and its mother with compatible St-negative blood the hospital pathologist (McCall) handled in a masterly fashion a new and unknown Rh blood-group situation.]

REFERENCES

Race, R. R. & Taylor, G. L. (1943) *Nature*, **152**, 300

Race, R. R., Taylor, G. L., Boorman, K. E. & Dodd, B. E. (1943) *Nature*, **152**, 563

Race, R. R., Taylor, G. L., Cappell, D. F. & McFarlane, M. N. (1944) *Nature*, **153**, 52

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THE INCIDENCE OF HÆMOLYTIC DISEASE OF THE FŒTUS ("ERYTHROBLASTOSIS FŒTALIS") IN DIFFERENT FAMILIES: the Value of Serological Tests in Diagnosis and Prognosis

by K. E. Boorman, B. E. Dodd & P. L. Mollison, *Journal of Obstetrics and Gynæcology of the British Empire*, **51**, 1–23, February 1944

This paper reports on a large amount of work on hæmolytic disease of the newborn done at the South-West London Blood Supply Depot.

The authors applied certain pathological criteria before they accepted the diagnosis of hæmolytic disease. The results are given of the serological investigation of the 100 families that qualified. Of the mothers 97 were Rh-negative, and 93 of these had anti-Rh in their serum. Of the 3 Rh-positive mothers 1 had the anti-Rh_2 agglutinin and the other 2 had anti-B agglutinins of unusual strength, to which the authors attributed the hæmolysis. This suggests that, when the diagnosis of hæmolytic disease is made with certainty, anti-Rh is responsible for an even higher proportion of cases than was thought before, almost all of them in fact.

The titre of anti-Rh agglutinin was measured at intervals throughout 7 pregnancies. In 2 the titre rose and in 3 remained steady before the birth of an affected child. In 2 it remained steady and Rh-negative children were born. The authors usually found a rise of titre after delivery, 8–20 days post-partum being the peak and the favourable time to search for agglutinins.

A group of 60 infants with physiological jaundice was investigated. Six of their mothers were Rh-negative and 54 Rh-positive, figures which suggest that Rh plays but little part in this type of jaundice. However anti-Rh was found in the serum of three of the six mothers. One of these mothers had previously had a child which suffered from hæmolytic disease, so it is likely that the jaundice of the present infant was not physiological. There was no excess of heterospecific pregnancies on the ABO system in this group. The authors conclude: "There is, therefore, no evidence that 'physiological jaundice' of the newborn is, in the great majority of cases, in any way connected with the presence of incompatible agglutinins in the mother's serum."

The diagnostic value of serological tests when hæmolytic disease is suspected is summed up thus: (i) The diagnosis is very probable when the mother is Rh-negative and has anti-Rh in her serum, or when she is Rh-positive and has any other atypical agglutinin acting on her baby's cells at 37° C. (ii) The diagnosis is less probable when the mother has very strong anti-A or anti-B agglutinins incompatible with her baby's cells, or when the mother is Rh-negative but no anti-Rh can be found. (iii) The diagnosis is improbable when the mother is Rh-positive and there is no atypical agglutinin in her serum, or when her anti-A and anti-B are not excessively strong.

Concerning the value of serological tests in prognosis, it is pointed out that the main importance of routine Rh tests in pregnancy is to ensure that Rh-negative women will not be given Rh-positive blood should they be transfused. In families in which hæmolytic disease has already occurred the prognosis is made less gloomy if the father can be shown to be heterozygous by finding an Rh-negative amongst the children. [The direct testing for homozygosity was hardly developed when this paper was written.] In all cases where anti-Rh is found in the mother's serum arrangements should be made to have Rh-negative blood ready for both mother and baby.

There is also a discussion on the possible parts played in this disease by the three factors, titre of maternal agglutinin, placental permeability and amount of extracorpuscular group-specific antigen.

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HÆMOLYTIC DISEASE OF THE NEWBORN: THE PREPONDERANCE OF HOMOZYGOUS Rh-POSITIVE FATHERS

by G. L. Taylor & R. R. Race, *British Medical Journal*, **1**, 288–289, 26/2/44

The results reported in this paper from the *Medical Research Council* Serum Unit confirm the suggestion made by Race, Taylor, Cappell & McFarlane (1943), that there is a preponderance of homozygous (RhRh) fathers in families affected by hæmolytic disease of the newborn. An Rh-negative woman is more liable to be immunized when every pregnancy is Rh-positive and provides the antigenic stimulus, as happens with a homozygous husband, than when he is heterozygous (Rhrh) and some children may be positive and others negative. About 3 out of 7 Rh-positive males are homozygous and 4 heterozygous.

All bloods which are not agglutinated by St serum * are homozygous RhRh and are about half of all the homozygotes. As 18 St-negatives were found amongst the husbands of 46 Rh-negative mothers with sera containing anti-Rh agglutinins and children suffering from erythroblastosis fœtalis, the authors estimate that 36 husbands were homozygous and 10 heterozygous, instead of the 20 homozygotes and 26 heterozygotes expected in a random sample of 46 Rh-positive people.

By serological tests (Race & Taylor, 1943; Race, Taylor, Boorman & Dodd, 1943; Race, Taylor, Cappell & McFarlane, 1944), the Rh genotypes of some of the husbands were determined, and further evidence of the predominance of homozygous fathers was obtained. Thus, of 38 husbands, only 5 were Rh_1rh and 33 not Rh_1rh, instead of the expected 15 and 23. About 33 % of people are of the genotype Rh_1rh—that is about 40 % of Rh-positives, and the shortage of this commonest type of heterozygote is highly significant. More complete genotype tests were made on 18 of the husbands and it was shown that at least 13 were homozygous instead of the 7 expected in 18 Rh-positive people.

This predominance of homozygous fathers affects very greatly the chances that Rh-negative, and therefore unaffected, children will be borne by Rh-negative mothers who have already had affected children. The genotype of about three-quarters of Rh-positive people can be determined serologically, and unless it can be shown serologically, or by his having an Rh-negative child or parent, that the husband is heterozygous, the prognosis is very poor.

REFERENCES

Race, R. R. & Taylor, G. L. (1943) *Nature*, **152**, 300

Race, R. R., Taylor, G. L., Boorman, K. E. & Dodd, B. E. (1943) *Nature*, **152**, 563

[1] Race, R. R., Taylor, G. L., Cappell, D. F. & McFarlane, M. N. (1943) *Brit. med. J.* **2**, 289

Race, R. R., Taylor, G. L., Cappell, D. F. & McFarlane, M. N. (1944) *Nature*, **153**, 52

[1] [*see BMB* **426**]

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ERYTHROBLASTOSIS FŒTALIS

by J. R. Gilmour, *Archives of Disease in Childhood*, **19**, Part I: 1–11; Part II: 12–20; Part III: 21–25, March 1944

In these three papers from the Bernhard Baron Institute of the *London* Hospital the author describes in detail the macroscopic and microscopic findings in 52 fatal cases of erythroblastosis fœtalis. The papers include a comprehensive survey of previous literature.

The author classifies the present series into 3 types, described as (i) hydrops fœtalis (24 cases); (ii) icterus gravis (26 cases); (iii) congenital anæmia (2 cases). Evidence is given that the three types are varieties of a single disease.

There is a brief discussion at the end of the third paper of the relationship between erythroblastosis and the Rh factor. Nineteen excellent photomicrographs are reproduced.

435

AN 'INCOMPLETE' ANTIBODY IN HUMAN SERUM

by R. R. Race, *Nature*, **153**, 771–772, 24/6/44

The discovery of an "incomplete" antibody in certain anti-Rh sera indicates that there is still much to be learnt about this versatile system of antigens and antibodies. By an incomplete antibody is here meant one which will combine with its specific antigen on the erythrocyte, but which is not a suitable partner for the agglutination stage.

Human anti-Rh serum of the type called by Wiener "standard" agglutinates erythrocytes of the gene Rh_1 and also those of Rh_2. Anti-Rh_1 serum agglutinates the former

* [*see BMB* 431.]

but not the latter. If, however, cells of the genotype Rh_2Rh_2 or Rh_2rh are suspended in anti-Rh_1 serum, which causes no agglutination, and after a few minutes are separated from this serum, washed and re-suspended in saline, then these treated cells can no longer be agglutinated by standard anti-Rh serum. These treated cells are agglutinated as well as ever by anti-Rh_2 serum and by St serum. In other words only one of the three antigens which must be present on Rh_2 cells is being blocked, and the other two are left free and ready for agglutination. This interpretation was supported by absorption experiments. The inhibited antigen is that which would normally react with the standard anti-Rh antibody, and the author considers the inhibiting agent to be an incomplete form of this antibody.

Five out of six anti-Rh_1 sera contained the incomplete antibody in good strength, the sixth in weak but definite amount. One standard anti-Rh serum contained the incomplete as well as the complete form of the antibody. In such a natural mixture, or in one artificially made, the incomplete antibody wins the race for antigen when erythrocytes are added, and the expected agglutination does not occur.

A brief account is given of R. A. Fisher's formulation of the relationships found in the rhesus factor.

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SYMPTOMLESS INCOMPATIBLE TRANSFUSION AND RESULTANT CHANGES IN ISO-AGGLUTININ TITRE

by R. Drummond, *British Medical Journal*, **1**, 483–485, 8/4/44

Incompatible blood transfusions in which studies of iso-agglutinin titres have been made are not numerous. In this paper the Transfusion Officer of the Welsh Board of Health reviews the cases reported previously and records another.

The agglutinogens of the ABO system of blood groups are regularly antigenic for man, and when cells containing A or B are transfused into a recipient whose serum contains the corresponding agglutinin, there is usually an initial fall in the strength of the agglutinin followed, within a week or ten days, by a rise, sometimes to enormous heights. The strength of the patient's reaction to the incompatible transfusion varies from being so weak as to pass unobserved to being strong enough to kill.

A male patient aged 54, of blood group B, was inadvertently transfused at a very slow drip rate over a period of 5½ hours with about 460 cm.³ of seven days old stored blood of group A. The transfusion was stopped as soon as it was noticed that the blood was labelled group A. The patient had complained of no symptoms whatever, and there had been no rise of temperature or pulse rate. Six hours later the pulse rose to 102 and the temperature to 100° F. [about 38° C.] but the patient still felt well. Within 12 hours the temperature was normal and remained normal or sub-normal. In 24 hours the pulse dropped to 76. He had occasional pyrexia before the transfusion. No serious complications developed and the patient's condition remained satisfactory. He was observed for several months.

Examination of blood taken from the patient on the day before the incompatible transfusion and of the conserved erythrocytes of the donor confirmed that the recipient was group B and the donor group A. The anti-A titre of the patient's serum was 100. On the third day after the transfusion it was 16, and thereafter rose rapidly and attained a peak of 25,600 on the fifteenth day. The titre fell slowly, and eight months later was not quite back to its pre-transfusion level. In the blood taken on the third day, agglutinates of 20 to 30 erythrocytes were seen lying free among large numbers of unagglutinated cells; on the fourth day there were a few small agglutinates but none on the fifth day.

In an attempt to find an *in vitro* explanation for the absence of symptoms, the sera of the donor and recipient were tested for agglutinin strength and their erythrocytes for sensitivity to iso-agglutination. These tests were made eight months after the incompatible transfusion when the strength of the recipient's anti-A agglutinin was approximately what it had been just before the transfusion. The group A donor's serum did not contain very powerful anti-B, and the author suggests that this agglutinin in no way harmed the recipient's cells and that absence of symptoms may in part be attributed to this. The recipient's anti-A agglutinin was of just less than average strength, whilst both donor and recipient erythrocytes were of average sensitivity. The agglutinogens A and B are not confined to erythrocytes, but occur also in the plasma. Thus the plasma of a group A person contains A agglutinogen. *In vitro* tests provided no evidence that the recipient's plasma protected its erythrocytes from the donor's iso-agglutinins and vice versa.

The author concludes that the *in vitro* tests did not fully explain the absence of symptoms and suggests the absence may have been due to the incompatible blood being given at a very slow drip rate.

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ON THE INHERITANCE AND LINKAGE RELATIONS OF ACHOLURIC JAUNDICE

by R. R. Race, *Annals of Eugenics*, **11**, 365–384, December 1942

This paper reports a search for linkage between the gene for acholuric jaundice and the common " physiological " genes, carried out at the Galton Laboratory, University College, London and the British Postgraduate Medical School, Hammersmith.

Nearly 200 members of 26 families were examined hæmatologically for the signs of acholuric jaundice by Dr. Janet Vaughan and Miss Olive Booth. They were also tested for the A_1A_2 BO and MN blood groups and for the ability to secrete the ABO antigens in the saliva, and to taste phenylthiocarbamide. Eye colour and freeness or attachment of the ear lobes were also scored, although the manner of inheritance of these characters is not on the sure basis of those mentioned before.

The war brought the investigation to an end, but not before evidence had been collected against partial sex-linkage and against close autosomal linkage with any of the genes responsible for the A_1A_2BO blood groups, the MN blood groups, secretion of the ABO antigens in the saliva, eye colour and attachment of ear lobes. The figures for taste-testing were inconclusive.

A year or so after the close of the investigation the Rh groups were discovered. This indicates what a laborious undertaking the great work of mapping the human chromosomes will be.

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THE GROUP-SPECIFIC SUBSTANCES, A, B, M, N AND Rh: THEIR OCCURRENCE IN TISSUES AND BODY FLUIDS

by K. E. Boorman & B. E. Dodd, *Journal of Pathology and Bacteriology*, **55**, 329–339, July 1943

That the group-specific substances A and B occur not only in erythrocytes but in almost all tissues and body fluids has been known for years. Attempts to demonstrate the erythrocyte agglutinogens M and N in tissues and body fluids were unsuccessful—save that Zacho found them sometimes in malignant tumours—until Kosjakov & Tribulev (1940), by the use of a modified inhibition technique, detected them in non-malignant tissues. As the addition of saliva to anti-Rh sera did not affect the agglutinin, American workers have concluded that the recently discovered Rh antigen is absent from saliva.

The present authors, of the South-West London Blood Supply Depot (*Medical Research Council*), have demonstrated the presence of M, N and Rh group-specific substances in tissues and body fluids. To detect Rh their technique was essentially the same as the inhibition technique which is used to detect A and B; it depends on the reduction in agglutinin strength which takes place when an antiserum is mixed with a substance containing the corresponding antigen. The reduction is measured by comparing the titre of a serum before and after mixture with the antigen. Every attempt to detect M and N by the inhibition technique failed because non-specific absorption was so great: that is, substances which contained only M would absorb not only anti-M specifically, but also anti-N non-specifically and vice versa. When the inhibition technique was modified according to suggestions made by Kosjakov & Tribulev for eliminating error due to non-specific absorption, M and N were disclosed in saliva and in tissues as these workers had described. The modified technique took account of Kosjakov & Tribulev's

finding that M group-specific substance saturated with anti-M serum cannot affect either anti-M or anti-N sera, but saturated with anti-N serum it can still specifically inhibit anti-M serum. Similarly, N substance saturated with anti-N serum can inhibit neither anti-M nor anti-N, but saturated with anti-M it inhibits anti-N.

Boorman & Dodd believe that earlier workers failed to find M and N in tissues because they could not deal with non-specific absorption and because they used extracts rather than suspensions of tissues. M and N seem to be only slightly soluble in water, for very little can be detected in saliva, and alcohol is said to destroy them. So neither aqueous nor alcoholic extracts would be likely to contain M or N. Whilst there are considerable amounts of Rh substance in the tissues, very little can be found in saliva, probably because it is almost insoluble in water.

The authors suggest that the Rh group-specific substances in fœtal tissues fail to neutralize maternal anti-Rh and prevent hæmolytic disease of the newborn because the neutralization of agglutinins which cross the placenta from the maternal to the fœtal circulation is effected by antigens in the body fluids, and that tissue antigens play little, if any, part. Similarly, in the few cases in which the ABO groups may cause hæmolytic disease and the baby probably belongs to the "non-secretor" class, the maternal agglutinin is not neutralized because the antigen is absent from the body fluids. Hartmann (1938) has shown that while the ABO antigens occur in the tissues of both secretors and non-secretors, and are present in alcohol- and water-soluble forms in secretors, they do not occur in water-soluble form in non-secretors, and are therefore almost completely absent from their body fluids. Boorman & Dodd conclude that if the following two conditions are satisfied hæmolytic disease of the newborn may result: (i) an incompatibility between the maternal serum and the fœtal erythrocytes, and (ii) an insufficiency of the corresponding antigen in the body fluids of the fœtus.

REFERENCES

Hartmann, G. (1938) *Z. ImmunForsch.* **93,** 385

Kosjakov, P. N. & Tribulev, G. P. (1940) *J. Immunol.* **37,** 297

Anæmias and their Treatment

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NUTRITIONAL IRON DEFICIENCY ANÆMIA IN WARTIME. I. The Hæmoglobin Levels of 831 Infants and Children

by L. S. P. Davidson, G. M. M. Donaldson, M. J. Dyar, S. T. Lindsay & J. G. McSorley, *British Medical Journal*, **2,** 505–507, 31/10/42

In this paper, workers in the Department of Medicine of Edinburgh University report the results of a survey of hæmoglobin levels in 442 infants and pre-school children (318 from birth to 23 months; 124 from 2 to 4 years) and 389 school-children of 5 to 12 years. The Haldane hæmoglobinometer (13·8 g./100 cm.^{3} equalling 100%) was used throughout the investigation, and all apparatus was checked against a standard set.

Results were compared with those obtained in a similar investigation made a few years before on Aberdeen children of the same age groups (Davidson & Campbell, 1935). Graphic representation of the results in both investigations shows a close parallelism in the curves of hæmoglobin percentages at different ages. From the 4th month there is a gradual fall which becomes more acute from the 8th to the 11th months. There is then a gradual rise, more rapid in the Edinburgh series, until 2 years of age. From 2 to 5 years, there is a progressive rise in hæmoglobin percentage, with higher values in the Aberdeen series. From 5 to 12 years, the rise is continued, with fluctuations, and comparison of the two curves shows that the hæmoglobin level was considerably lower in the Edinburgh series (average difference of 10% for all ages in this group). The Edinburgh children had no obvious signs of malnutrition.

Analysis of the figures in these two and in a third (Wills, Mackay, Bingham & Dobbs, 1942) series showed that none of the Aberdeen (1935) children had a hæmoglobin of less than 80%, whereas almost half the Wills *et al.* (1942) and more than half of the Edinburgh (1942) children had hæmoglobin concentrations below that level.

The conclusions drawn from this study were that (i) after three years of war there was no significant change in infant hæmoglobin levels; (ii) there was a significant fall among children of school age; (iii) there should be provision of school meals of high iron content, and propaganda to encourage the consumption of iron-rich foods by children of school age.

REFERENCES

Davidson, L. S. P. & Campbell, R. M. (1935) *Brit. med. J.* **2,** 195

[1] Wills, L., Mackay, H. M. M., Bingham, K. & Dobbs, R. H. (1942) *J. Hyg., Camb.* **42,** 505

[1] [*see BMB* **441**]

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NUTRITIONAL IRON DEFICIENCY ANÆMIA IN WARTIME. II. The Hæmoglobin Levels of 3,338 Persons from Birth to 55 Years of Age

by L. S. P. Davidson, G. M. M. Donaldson, S. T. Lindsay & J. G. McSorley, *British Medical Journal*, **2,** 95–97, 24/7/43

In a previous paper (Davidson, Donaldson, Dyar, Lindsay & McSorley, 1942) investigations on hæmoglobin levels in 442 infants and pre-school children and 389 schoolchildren were reported. Since the publication of this paper, the *National Physical Laboratory* provided correction factors for the Haldane hæmoglobinometer used, which indicated that the reported readings were approximately 2% low. A further 121 infants and pre-school children, 633 school-children, and 1753 adolescent and adult males and females have been examined, making a total for the whole series of 3338 individuals. Of these, 563 were infants and pre-school children (birth to 4 years); 917 municipal primary-school children (5 to 12 years); 105 private-school children (5 to 12 years); 518 adolescent males and females (13 to 19 years); 620 adult males and females (20 to 54 years); 45 multiparous women; 570 pregnant women. All readings were adjusted in accordance with the correction factors.

The standard of normality for the groups, birth to 4 years, was considered as 85% ± 5% (Haldane); for the groups 5 to 12 years, 12 to 18 years, and for adult females, 95% ± 5% (Haldane); for adult males 100% ± 5% (Haldane) and for pregnant women 85% ± 5% (Haldane). "Clinical anæmia" was considered to be present if the hæmoglobin was below 80% (Haldane) for all groups except the group of pregnant women, where 70% (Haldane) was the arbitrary figure chosen. Using this standard, "clinical anæmia" was found to be present in 39% of municipal primary-school children, approximately 5% of private-school children, 12% of adolescent females, 7% of adult females, 24% of pregnant women, and less than 1% of adolescent and adult males.

The main conclusion (made with some reservations) from this study is that the incidence of "clinical anæmia" in Edinburgh municipal school children is significantly higher than it was in comparative Aberdeen children a few years before the war (Davidson & Campbell, 1935). Standards of normality and errors of estimation are discussed.

REFERENCES

Davidson, L. S. P. & Campbell, R. M. (1935) *Brit. med. J.* **2,** 195

[1] Davidson, L. S. P., Donaldson, G. M. M., Dyar, M. J., Lindsay, S. T. & McSorley, J. G. (1942) *Brit. med. J.* **2,** 505

[1] [*see BMB* **439**]

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ANÆMIA IN WOMEN AND CHILDREN ON WARTIME DIETS

by L. Wills, H. M. M. Mackay, K. Bingham & R. H. Dobbs, *Journal of Hygiene*, **42,** 505–526, October 1942

The investigation reported in this paper was undertaken to determine whether (i) anæmia was prevalent among women and children; (ii) there was any evidence of increased incidence of anæmia; (iii) there was any evidence as to the ætiology of the anæmia. Preliminary details of the investigation had previously been published (Mackay, Wills, Dobbs & Bingham, 1942). Haldane's hæmoglobinometer (13·8 g./100 cm.^{3}

equalling 100%) was used throughout the investigation. The estimations were made on 544 women (nurses and medical students; factory workers; housewives) and 530 children (364 aged 6 months to 5 years; 90 aged 5–13 years; 38 aged 13–15 years; 38 adolescent factory workers aged 14–18 years).

The mean hæmoglobin value of the women was 89·5% (ranging from 56%–120%), similar to the mean obtained by Davidson & Fullerton (1938) in 1937 for a group of poor nulliparous women. These figures are significantly lower than those obtained in other series by Price-Jones (1931) and Jenkins & Don (1933).

There was a significant difference in the hæmoglobin values for nurses and students working in London (90·6%) and in country (84·1%) hospitals. Mean values for the two different occupational groups (nurses and students) working at the same hospitals approximated closely. Mode of life and working conditions did not differ materially in the London and rural hospitals, but there were differences in the diet. The estimated daily iron intake of the London group was 10·8 mg. and that of the rural group 9·2 mg. The 159 workers at a factory in a small town near London had a higher (94·0%) mean hæmoglobin level than the London housewives (89·6%).

Of the children, the average hæmoglobin level at 6–12 months was 75·4%, dropping to 72·8% between 1–2 years, and gradually rising again to 81·8% between 4–5 years of age. In 90 children of the 5–13 year age-group, the mean hæmoglobin level was 80·3%; in the 13–15 year group it was 89·3% and in the 14–18 year group of working girls it was 98·7%.

In all the groups of women and children, except the small group of factory girls, mean hæmoglobin values were significantly lower than in previous comparable groups, and the authors believe these reductions to be the expression of a nutritional anæmia. They believe that iron deficiency is one important ætiological factor, but that other dietetic deficiencies are implicated, and that in nurseries a high incidence of infection has increased the incidence and severity of an anæmia primarily due to iron deficiency.

REFERENCES

Davidson, L. S. P. & Fullerton, H. W. (1938) *Edinb. med. J.* **45,** 1
Jenkins, C. E. & Don, C. S. D. (1933) *J. Hyg., Camb.* **33,** 36
Mackay, H. M. M., Wills, L., Dobbs, R. H. & Bingham, K. (1942) *Lancet*, **2,** 32
Price-Jones, C. (1931) *J. Path. Bact.* **34,** 779

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DISCUSSION ON NUTRITIONAL ANÆMIA IN CHILDREN AND WOMEN: A War-time Problem

by H. M. M. Mackay, L. Wills, R. H. Dobbs, K. Bingham, L. Findlay, H. M. Sinclair, L. S. P. Davidson, J. Yudkin & T. S. Marshall, *Proceedings of the Royal Society of Medicine*, **36,** 69–84, December 1942

In the paper (Mackay, Wills, Dobbs & Bingham) which opened this discussion attention was drawn to previous observations (McCance, Widdowson & Verdon Roe, 1938; Ross & Summerfeldt, 1936; Spence, 1934) on the influence of economic status on mean hæmoglobin level and on the relationship between infantile anæmia and anæmia of the mother during gestation (Strauss, 1933; Mackay, 1933, 1935). There was evidence of a higher morbidity rate in babies with moderate anæmia (Mackay & Goodfellow, 1931). After a reference to fallacies due to obtaining samples of blood for hæmoglobin estimation from the ears of young children, comparisons are made between the results of different hæmoglobin surveys undertaken in Britain. Curves of mean hæmoglobin levels in groups of working-class children under 5 examined before the war by different British investigators (Davidson, Fullerton & Campbell, 1935; Fullerton, 1937; Hutchison, 1938) show close agreement and correspond also to the later war-time series of Wills, Mackay, Bingham & Dobbs (1942). There was also a close similarity between the war-time mean levels of Wills *et al.* for children in or near London and those of Davidson, Donaldson, Dyar, Lindsay & McSorley (1942) for Edinburgh. Hæmoglobin levels of women were significantly lower than those of comparable groups before the war.

From examination of evidence discussed in this paper it appears probable that there has been a reduction of hæmoglobin levels in some social groups, and it is possible, although not demonstrated, that there has been a rise in other groups as a result of increased employment, rationing and food "priorities."

The authors conclude by recommending that infants should be given iron supplements and that, for older groups, iron should be added to bread.

Another speaker, Dr. L. Findlay, in discussing the blood as an index of health, referred to the results of a survey (Findlay, 1937) undertaken on children admitted to a London hospital in a poor district. These hæmoglobin estimations were made from ear-punctures, and results may have been too high, but a subsequent unpublished series made from finger- and heel-punctures showed no evidence of a serious incidence of anæmia. Later observations made in war-time on children admitted to the *Radcliffe Infirmary* from Oxford and its environs revealed a high proportion of subnormal hæmoglobin and erythrocyte levels, although the general condition of the Oxford children appeared to be superior to that of the London children. The author discusses these and other relevant findings and concludes that the hæmoglobin level is not a true index of the health of the child, nor necessarily of the iron content of the diet; that it varies with age, sex, and other factors; and that the term "nutritional anæmia" is too loosely employed.

Dr. H. M. Sinclair referred to observations made in the course of the Oxford Nutrition Survey which showed an increase in anæmia. If this were due to iron deficiency, a simple remedy would be the use of iron instead of aluminium cooking pots. A potato boiled in an iron vessel contains about twice as much iron as one cooked in an aluminium vessel. With apples boiled for only 5 minutes, the iron content is nearly a hundred times greater if an iron vessel is used. Another simple measure is the provision of ferrated bread. In the author's view, there was no danger of toxic effects from excessive intake of iron.

Dr. R. H. Dobbs discussed anæmia in young women, with reference to the results reported by Wills *et al.* (1942), and sources of error in hæmoglobinometry. It was particularly important to obtain freely flowing blood from a warm limb.

Professor L. S. P. Davidson summarised results of a hæmoglobin survey which has been reported elsewhere (Davidson *et al.*, 1942).

Dr. J. Yudkin had recently examined 300 mothers and children in Scotland and had found a decrease in hæmoglobin levels as compared with previously published figures, although this was not so great as that found by Professor Davidson. It was important that all workers on this problem should maintain close contact and compare methods and results.

Dr. T. S. Marshall reported results of estimations on male and female blood donors. The mean hæmoglobin level (Haldane-Gowers hæmoglobinometer, standardised by National Physical Laboratory) was 100% in 100 males; 84% in 65 post-menopausal women; 82% in 220 women in the reproductive period of life. Differences as great as 12–18% were observed in the same subjects (*a*) immediately on arrival, after cycling or hurrying, (*b*) after 20 minutes recumbency. This suggested a possible reason for the high levels reported by Wills *et al.* (1942) in factory girls.

REFERENCES

[1] Davidson, L. S. P., Donaldson, G. M. M., Dyar, M. J., Lindsay, S. T. & McSorley, J. G. (1942) *Brit. med. J.* **2,** 505
Davidson, L. S. P., Fullerton, H. W. & Campbell, R. M. (1935) *Brit. med. J.* **2,** 195
Findlay, L. (1937) *Brit. med. J.* **1,** 12
Fullerton, H. W. (1937) *Arch. Dis. Childh.* **12,** 91
Hutchison, J. H. (1938) *Arch. Dis. Childh.* **13,** 355
McCance, R. A., Widdowson, E. M. & Verdon Roe, C. M. (1938) *J. Hyg., Camb.* **38,** 596
Mackay, H. M. M. (1933) *Arch. Dis. Childh.* **8,** 221
Mackay, H. M. M. (1935) *Lancet*, **1,** 1431
Mackay, H. M. M. & Goodfellow, L. (1931) *Spec. Rep. Ser. med. Res. Coun.* No. 157, 25
Ross, J. R. & Summerfeldt, P. (1936) *Canad. med. Ass. J.* **34,** 155
Spence, J. C. (1934) *Investigations into the health and nutrition of certain of the children of Newcastle-upon-Tyne*, Newcastle
Strauss, M. B. (1933) *J. clin. Invest.* **12,** 345
[2] Wills, L., Mackay, H. M. M., Bingham, K. & Dobbs, R. H. (1942) *J. Hyg., Camb.* **42,** 505

[1] [see *BMB* **439**] [2] [see *BMB* **441**]

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TREATMENT OF ANÆMIA IN SCHOOL-CHILDREN WITH IRON AND ASCORBIC ACID

by L. S. P. Davidson & G. M. M. Donaldson, *British Medical Journal*, **1**, 76–77, 15/1/44

In the first school, the children in both the control and the experimental groups were unselected, but were divided into three groups. Group A consisted of children who went home for a midday meal; Group B included those who took school dinners, for which they paid; while Group C included those children who had free school dinners. The children in the experimental groups were given 3 grains [about 200 mg.] of ferrous sulphate in tablet form daily for 5 days in each week. Hæmoglobin estimations were made at the outset of the experiment and again after 3 months of treatment.

In the control groups there was no significant rise in the mean hæmoglobin levels at the second examination. In experimental Groups B and C, the rise in the hæmoglobin levels was significant but in Group A, although there was a rise, it was not statistically significant. An analysis of the hæmoglobin figures of those children whose initial hæmoglobin level was less than 80 % showed that of 59 such children in the control series, 31 (52·5 %) still had hæmoglobin figures of less than 80 % at the second examination; while of 71 receiving iron therapy, only 24 (33·8 %) still had hæmoglobin figures of less than 80 % at the second examination. This improvement in the treated group is statistically significant.

In the second school there were 57 unselected children in the control group, 56 unselected children in experimental Group A who were treated with 25 mg. of ascorbic acid daily for 5 days a week, and 57 unselected children in Group B who were given 25 mg. of ascorbic acid daily and 3 grains of ferrous sulphate daily for 5 days a week. In the control group and in Group A there was no significant rise in the hæmoglobin values when the estimations were repeated at the end of 3 months. In Group B the rise in hæmoglobin level was significant.

In the third school, 54 children whose original hæmoglobin level was 80 % or less were divided in two equal groups, the experimental group being given 25 mg. of ascorbic acid daily and 6 grains of ferrous sulphate daily for 5 days a week over 6 months. Both control and experimental groups showed an increase in hæmoglobin but the mean levels in the treated group were significantly greater than those found in the control group. These results show that the hypochromic anæmia of these children was mainly conditioned by an insufficiency of iron in the diet and that supplement of 25 mg. of ascorbic acid daily had no effect in raising the hæmoglobin levels.

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TREATMENT OF PERNICIOUS ANÆMIA WITH AN EXPERIMENTAL PROTEOLYSED LIVER PREPARATION: Preliminary Observations

by L. J. Davis, L. S. P. Davidson, D. Riding & G. E. Shaw, *British Medical Journal*, **1**, 655–656, 29/5/43

The superiority of whole liver over parenteral injections of liver extracts in the treatment of certain types of macrocytic anæmia, such as those occurring in pregnancy, sprue and tropical nutritional deficiency states is well known. Among the disadvantages of whole liver are its relatively high cost, the difficulty of ensuring a regular supply, the distaste caused by its prolonged administration, and the uncertainties attending its assimilation in patients whose digestive and absorptive processes are impaired. Accordingly it was considered that it might be an advantage to administer to such patients whole liver in a soluble predigested form. Papain was chosen as a suitable enzyme for predigestion of the liver and the following method of preparation was used:

Take 330 pounds [about 150 kg.] of minced liver in a steam-heated pan. Add 36 gallons [about 160 l.] of water and 660 g. of papain, and heat the mixture to 60° C. Maintain the temperature at this level for three hours. Then raise the temperature to 100° C. and boil vigorously for ten minutes. Strain the hot digestion mixture through cloth, and concentrate the filtrate at a low temperature in a vacuum still to 20 gallons. Clarify the concentrate by filtration, using sterilized kieselguhr as a filter aid, and then sterilize by filtration through suitable asbestos pads. Dry the sterile liquid in a low-temperature vacuum oven. The product thus obtained is a dry, sterile, granular light-brown powder which is slightly hygroscopic. It is completely soluble in hot or cold water, giving a solution that is reasonably palatable and free from the characteristic liver flavour. Riboflavin is present in a concentration of 10 mg. per 100 g.

Five typical cases of pernicious anæmia were used to test this product. Without exception a satisfactory response was noted within a few days of the institution of treatment. The average rise in erythrocytes per mm.^{3} was 0·94 million during the first two weeks and 1·74 million during the first three weeks of treatment. An adequate daily dose appeared to be 2 drachms [about 4 g.], but in certain resistant cases it is possible that a larger amount may be desirable. This amount is derived from less than two ounces [about 60 g.] of raw liver.

[*This proteolysed liver preparation is now commercially available under the trade name "Hepamino."*]

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SENSITIVITY TO LIVER EXTRACT

by J. G. McSorley & L. S. P. Davidson, *British Medical Journal*, **1**, 714–716, 27/5/44

A search of the literature since Schlesinger (1930) first reported reactions to liver extract has revealed less than a hundred cases. Reactions may be classified as: (i) primary, which occur after the first injection, and have become rare with the increasing purity of liver preparations and adoption of routes of administration other than intravenous; (ii) secondary, which occur after varying periods of oral, intravenous or intramuscular liver therapy and are undoubtedly of an allergic character.

The literature contains reports of sensitivity to all brands of liver extract. Some patients who have reacted to one brand do not react to another. However, such patients tend to become sensitized also to alternative brands, and the experience of the authors indicates that sensitivity usually extends to all liver extracts in patients who have had marked reactions to one preparation. Reactions usually increase in severity with successive injections. The commonest are: flushing, tachycardia, erythema, and localized urticaria. In severe cases, there may be generalized urticaria, bronchospasm, vomiting, rigor, hyperpyrexia, collapse, angioneurotic œdema and other allergic manifestations.

The authors found skin tests to be of some value in assessing sensitivity, although they agree that their value is limited. Their technique is to inject intradermally 0·05 cm.^{3} of undiluted liver extract into the volar surface of the forearm, with a control injection of saline at the same level. A wheal of 15 mm. or more in diameter developing in 15–30 minutes is accepted as a positive reaction. As there may be severe general reactions to this test dose, adrenaline should be available.

With regard to treatment, patients were arbitrarily divided into those with (i) mild symptoms and (ii) severe constitutional reactions. In mild cases 3 minims [about 0·174 cm.^{3}] of 0·1 % adrenaline hydrochloride were injected concurrently with the liver preparation and in some cases no further treatment was required. In other cases the quantity of liver extract injected was reduced to $\frac{1}{2}$ or $\frac{1}{4}$ of the dose which produced a reaction and the periods between injections were correspondingly reduced. Other measures have been recommended by various writers but proof of their value is lacking. It has been claimed (Barfred, 1942) that butyl alcohol removes a considerable part of the factor in liver extract responsible for allergic reactions, but the present authors found that material so treated produced reactions in 2 sensitive patients.

In severe cases oral ingestion of liver or hog-stomach preparations should replace parenteral liver therapy, or desensitization should be carried out. A technique of desensitization which was found satisfactory in 17 severe cases is described. The authors prefer desensitization to substitution of oral preparations, as parenteral therapy is cheaper and more effective: Desensitization, however, may be dangerous unless performed by someone experienced in the danger of anaphylactic reactions.

REFERENCES

Barfred, A. (1942) *Acta med. scand., Suppl.* **131**

Schlesinger, W. (1930) *Wien. med. Wschr.* **80**, 696

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AUTOLYSED YEAST IN THE TREATMENT OF NUTRITIONAL MACROCYTIC ANÆMIA

by G. R. Sippe, *British Medical Journal*, **1**, 656–658, 13/5/44

Nutritional macrocytic anæmia has been reported from India, China, Africa, and Macedonia, and has been attributed to the lack of one or more essential factors in the diet. In this paper from the Bacteriological Laboratory of Reduit, Mauritius, the author states that the condition is also common in Mauritius among those who refrain on religious grounds from eating beef flesh. Wills (1934) showed autolysed yeast to be active in the treatment of this anæmia. The present author reports the results in 6 cases treated by oral administration of a waste product of the manufacture of alcohol by fermentation of molasses. Its potency was attributed to the presence of autolysed yeast. The product was obtained as a thick fluid from the bottom of the fermentation vats. Before use, it was passed through a coarse filter and heated at 70° C. for one hour. The quantity of the product used in treatment was 3–6 ounces (about 90–180 cm.^{3}) daily. Results of the analysis of samples are tabulated.

REFERENCE

Wills, L. (1934) *Indian J. med. Res.* **21**, 669

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HÆMOLYTIC ANÆMIAS

by L. J. Davis, *Edinburgh Medical Journal*, **50**, 589–616, October 1943

This is the text of a Honeyman Gillespie Lecture, in which there is a brief consideration of fundamental principles of erythrocyte destruction and regeneration, followed by a survey of the different types of hæmolytic anæmia in the light of recent views and observations. The author adopts a provisional classification of the hæmolytic disorders according to their known or supposed ætiology:

i. *Abnormality of the erythrocytes*
   - Familial acholuric jaundice
   - Sickle-cell anæmia
   - Nocturnal hæmoglobinuria

ii. *Hæmolysins*
   - Transfusion incompatibility
   - Erythroblastosis fœtalis
   - Paroxysmal hæmoglobinuria
   - Severe burns
   - Bacterial infections
   - Snake bite

iii. *Parasitic infection of erythrocytes*
   - Malaria
   - Oroya fever

iv. *Poisons*
   - e.g. lead, phenylhydrazine, saponin

v. *Hypersensitivity to drugs or other agents*
   - Sulphonamide anæmia
   - Fabism
   - Baghdad Spring anæmia

vi. *Cause unknown, but associated with known predisposing factors*
   - Blackwater fever (sub-tertian malaria)
   - "Symptomatic" hæmolytic anæmias (Hodgkin's disease; reticuloses; carcinoma, etc.)
   - March hæmoglobinuria (postural defect)

vii. *Cause completely unknown*
   - Acute hæmolytic anæmia (so-called "Lederer's anæmia")
   - Miscellaneous subacute and chronic hæmolytic anæmias
   - Cooley's (Mediterranean) anæmia

Each group of hæmolytic disorders is discussed under the above headings, and 108 references to the literature are given.

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SYMPTOMATIC HÆMOLYTIC ANÆMIA: A REPORT OF FOUR CASES

by L. J. Davis, *Edinburgh Medical Journal*, **51**, 70–83, February 1944

The term symptomatic hæmolytic anæmia was introduced by Dameshek & Schwartz (1940) to embrace cases of acquired hæmolytic anæmia displaying an apparent ætiological relationship to certain morbid processes such as carcinomatosis, leukæmia, Hodgkin's disease, sarcoidosis, hepatitis, teratoma of the ovary, and various infective conditions such as tuberculosis and lobar pneumonia. Dr. Davis reports in detail 4 further cases in which the primary diseases were adenocarcinoma of the pancreas, reticulosis, Hodgkin's disease and acute myeloid leukæmia. The author stresses the need for exclusion of other types of hæmolytic anæmia before this diagnosis is accepted. Microspherocytosis and increased erythrocyte fragility may or may not occur. These were absent in the present cases, all of which were macrocytic to a greater or less extent.

The mechanism of hæmolysis is obscure. In cases where the marrow is extremely affected it is possible to postulate a disturbance of marrow function resulting in the production of abnormal erythrocytes having impaired survival values. The author suggests that the neoplastic process may exert an abnormal stimulus to increased activity of the reticulo-endothelial cells, and points to the infrequency with which the spleen becomes the site of carcinomatous metastases because of the ability of the numerous reticulo-endothelial cells of this organ to destroy wandering tumour cells. Thus it is conceivable that the constant exercise of such a destructive mechanism directed against tumour cells may result incidentally in an increased destruction of erythrocytes. However, this mechanism would not account for all cases. The prognosis and treatment necessarily depend upon the underlying conditions. All the 4 cases described died, but a few cases in the literature benefited by the removal of the original tumour and by splenectomy.

REFERENCE

Dameshek, W. & Schwartz, S. O. (1940) *Medicine*, **19**, 231

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ACUTE HÆMOLYTIC ANÆMIA: REPORT OF A CASE PRESENTING HITHERTO UNREPORTED FEATURES

by J. P. Currie, *British Medical Journal*, **2**, 8–10, 1/7/44

This paper from the Royal Infirmary, Glasgow, gives a brief summary of the growth of knowledge of acute hæmolytic anæmia and reports a case of this rare condition showing auto-agglutination and auto-hæmolysis at room temperature. The reader is referred to the full review of the subject by Dameshek & Schwartz (1940).

Hayem (1898) and Minkowski (1900) first recognised hæmolytic jaundice as an entity apart from liver or bile-duct disease. Chauffard (1907) showed that in chronic hereditary acholuric icterus with splenomegaly the erythrocytes were abnormally fragile in hypotonic solution, and other workers described "acquired hæmolytic icterus," a type of acholuric jaundice which was not hereditary and occurred alone or in the course of other diseases. In a third type, "hæmolysinic icterus," the serum, at the height of the disease, contained an isohæmolysin. Each of the three types was subdivided into acute, subacute and chronic. Roth (1912) found an auto-agglutinin and an auto-hæmolysin in a case, but said that "hæmolysinic" was an unjustifiable term and that the hæmolysis was due to an abnormality of the erythrocytes. The existence of an acquired type was gravely doubted. In 1925 Lederer "discovered" the "new" disease, acute hæmolytic anæmia, although similar cases had been reported previously. He did not mention isohæmolysins or auto-agglutinins.

It is very difficult to trace cases because they may be given a variety of names, e.g. "atypical anæmia," "atypical pernicious anæmia," "acute pernicious anæmia," etc.

Whilst many hæmolytic anæmias are due to bacteria, protozoa and chemicals, no cause is known for the majority of cases. There are two main views:

(i) that the serum or tissues contain an auto-hæmolysin for the patient's own erythrocytes.

(ii) that lysis is caused by normal wear of abnormally fragile erythrocytes.

That the marrow responds so well suggests it is not injured and favours the first view.

Donath & Landsteiner (1905) showed that the lysis in paroxysmal hæmoglobinuria is caused by isohæmolysins and they have been found in several cases of acute hæmolytic anæmia, but with regard to the ætiology of the disease the

finding of auto-hæmolysins is of greater significance; these have rarely been demonstrable and only under abnormal conditions, e.g. at ice-box temperature. Early workers noted auto-agglutination in acute acquired hæmolytic jaundice, and said its occurrence was constant and of diagnostic value. This observation was for long overlooked and only recently re-made.

Dameshek and Schwartz made a hæmolysin against guinea-pig erythrocytes by injecting them into a rabbit, and then, by injecting varying amounts of the rabbit serum into guinea-pigs, were able to produce various types of hæmolytic syndrome—from subacute hæmolytic anæmia with small doses, to fulminating hæmolytic anæmia and hæmoglobinuria with large doses of the hæmolysin. The clinical conditions, spherocytosis, increased erythrocyte fragility, reticulocytosis, pseudo-macrocytosis, etc., could be produced in the same way, and it is inferred that the clinical syndromes are due to the amount of hæmolysin present.

The author describes the case of a man of 37 who became suddenly ill with acute hæmolytic anæmia, with lemon-yellow skin and mucous membranes, striking pallor, tender palpable spleen, and hæmoglobinuria. There was no bilirubinuria.

Blood-grouping preparatory to transfusion was made difficult by the patient's serum agglutinating his own erythrocytes at room temperature. By performing the tests at body temperature the patient's group was shown to be O. In addition to the normally occurring isoagglutinins anti-A and anti-B, the patient's serum contained an agglutinin which at room temperature—not at body temperature—agglutinated his own corpuscles but not those of normal group O persons—it was literally an auto-agglutinin. [Usually "auto-agglutinin" is used for the commonest type of cold agglutinin which agglutinates not only the corpuscles of its donor but those of any other person.] Heating to 60° C. did not destroy the antibody and the addition of complement caused hæmolysis. [By saying "The associated hæmolysin requires the presence of complement" the author suggests that the agglutinin and the hæmolysin are not the same antibody. Hæmagglutination and hæmolysis are closely related and it is widely believed that the addition of complement causes the agglutinin to lyse the erythrocytes.] Standing normal group O cells in the filtrate from the patient's crushed, washed erythrocytes made them sensitive to the auto-agglutinin—it was impossible to determine whether the corpuscles, suspended in a hæmoglobiniferous fluid, were also lysed. After blood transfusion recovery took place, the auto-agglutination and auto-hæmolysis disappeared, and serum obtained whilst he was ill did not affect his corpuscles after recovery, but these corpuscles treated with the filtrate mentioned above were rendered agglutinable. After recovery the serum still contained the auto-agglutinin—it agglutinated cells obtained whilst he was ill. Recovery caused an alteration in the erythrocytes, not in the serum. It is concluded that there were: (*a*) an auto-agglutinin, an auto-agglutinogen and an auto-hæmolysin; and it is suggested there was also (*b*) an auto-hæmolysinogen.

Only one pint [about 570 cm.³] of citrated group O blood was given on the sixth day of the illness, but the author does not regard it as a case of dramatic recovery due to a single transfusion—the disease had limited itself by the time the blood was given. Several transfusions may be needed and in some cases they may be useless. Splenectomy may cure some, and it is thought that the hæmolysin is made in the spleen.

REFERENCES

Chauffard, M. A. (1907) *Sem. méd., B. Aires*, **29**, 47
Dameshek, W. & Schwartz, S. O. (1940) *Medicine*, **19**, 231
Donath, J. & Landsteiner, K. (1905) *Z. klin. Med.* **58**, 173
Hayem, G. (1898) *Pr. méd.* **1**, 121
Lederer, M. (1925) *Amer. J. med. Sci.* **170**, 500
Minkowski, O. (1900) *Verh. Kongr. inn. Med.* **18**, 316
Roth, O. (1912) *Dtsch. Arch. klin. Med.* **106**, 137

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THE DESTRUCTION OF TRANSFUSED ERYTHROCYTES IN ANÆMIA

by G. M. Brown, O. C. Hayward, E. O. Powell & L. J. Witts, *Journal of Pathology and Bacteriology*, **56**, 81–94, January 1944

The authors applied the Ashby technique of differential agglutination to the study of the survival of transfused erythrocytes in different types of anæmia. Results were examined by construction of graphs showing the relation of the number of surviving donor cells to the days after transfusion. In the simple types, such as idiopathic hypochromic anæmia (6 cases), only a linear type of decay was found, but in hæmolytic anæmia (3 cases) the plotted results showed considerable curvature and the average life of the cell was much reduced. The method therefore allows a quantitative analysis of the amount of pathological hæmolysis in other anæmias. To facilitate numerical description of the results, a general equation connecting the number of surviving cells with the interval since transfusion was developed. Seven cases of anæmia complicating infections of varying severity were transfused, some on several occasions. These cases showed different rates of hæmolysis, and the average life of the transfused cells ranged from 8·4 to 56·3 days. The shape of the decay curves varied from a curvature as great as that seen in clinical hæmolytic anæmia, to a straight line such as had been found in the cases of idiopathic hypochromic anæmia. The cases which were judged more severe on clinical grounds showed more rapid hæmolysis and in the cases where the average life was shortest there was increased reticulocytosis and bilirubinæmia. Thus the anæmia which complicates acute infection is apparently due to unduly rapid erythrocyte destruction, but in chronic infections the destruction rate is normal. Abnormally rapid hæmolysis was also found in cases of puerperal pernicious anæmia, leuco-erythroblastic anæmia and myelomatosis, while in cases of thyrotoxicosis, chronic nephritis and pernicious anæmia under treatment with liver (1 case of each), erythrocyte destruction occurred at the normal rate.

In a discussion of their results, the authors relate the mathematical expression of their data to the hypothesis recently developed by Maegraith, Martin & Findlay (1943) that a (species-specific) lytic enzyme and an inhibitor are concerned in the mechanism of hæmolysis.

REFERENCE

[1] Maegraith, B. G., Martin, N. H. & Findlay, G. M. (1943) *Brit. J. exp. Path.* **24**, 58

[1] [*see BMB* **87**]

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STUDIES IN THE ANÆMIAS OF INFANCY AND EARLY CHILDHOOD. XII.—THE REGENERATION RATE OF HÆMOGLOBIN AND THE LIFE SPAN OF ERYTHROCYTES IN NORMAL AND PATHOLOGICAL CONDITIONS

by H. S. Baar & T. W. Lloyd, *Archives of Disease in Childhood*, **18**, 1–21, March 1943

Blood containing varying numbers of reticulocytes was incubated in sterile citrated balanced salt solutions, with or without added glucose or serum, and the rates of maturation of the reticulocytes were observed by hourly counts. The curve of maturation shows a decreasing velocity of disappearance of the reticulocytes. The authors consider that this is due to the initiation of necrobiotic changes in the erythrocyte and does not represent a real decrease of the velocity of maturation. The maturation time is therefore obtained by extrapolation of the initial slope to the ordinate zero. The average maturation time of normal reticulocytes by this method is about seven hours. The maturation time in cases with reticulocytosis is usually longer, but there is no fixed relation between either the number of reticulocytes or the amount of reticular substance and the maturation time. The only condition in which a maturation time shorter than the normal was found is untreated pernicious anæmia.

The daily regeneration rate is obtained by multiplying the number of reticulocytes per cent. by twenty-four and dividing by the maturation time in hours. One hundred divided by this figure gives the life span of erythrocytes in days if the number of erythrocytes is constant. From an average reticulocyte count of 0·7% and a maturation time of seven hours, an average life span of forty-two days has been deduced. The regeneration rate of hæmoglobin is identical with that of erythrocytes as long as the colour index remains unchanged.

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STUDIES IN THE ANÆMIAS OF INFANCY AND EARLY CHILDHOOD. XIII—EXPERIMENTS ON BLOOD REGENERATION AND THEIR SIGNIFICANCE FOR THE LIFE SPAN OF ERYTHROCYTES

by H. S. Baar, *Archives of Disease in Childhood*, **18**, 65–87, June 1943

A curve representing the rise of hæmoglobin or of erythrocytes does not indicate the real regeneration but is the result of two factors—the formation (or, more exactly, the release from the bone-marrow) of erythrocytes and their destruction. It was decided to separate these two factors in experimental anæmia, calculating the real regeneration or the daily hæmoglobin intake from the number of reticulocytes and their maturation time determined *in vitro*, and sometimes *in vivo*, and estimating the destruction, subtracting the observed rise of hæmoglobin from the intake determined in this way. Anæmia was produced by subcutaneous injections of phenylhydrazine hydrochloride in rabbits and guinea-pigs. The daily regeneration of hæmoglobin (= intake) per 100 cm.³ of blood was noted for two- or three-day periods, over some weeks. This figure is calculated by taking the arithmetic mean of two subsequent reticulocyte counts (as a percentage), dividing by 100 and multiplying by 24 over the maturation time in hours. The maturation was determined during the period of high reticulocyte counts for rabbits both *in vitro* and *in vivo*, for guinea-pigs *in vitro* only. These figures, which were 10 hours for rabbits and 8 hours for guinea-pigs, were used for the whole experimental period. The regeneration per 100 cm.³ blood is therefore given by the formula $\frac{Hb \times R}{100} \times 2{\cdot}4$ for rabbits and $\frac{Hb \times R}{100} \times 3$ for guinea-pigs, where Hb is hæmoglobin in grams per 100 cm.³ and R the reticulocyte count per cent. of erythrocytes. If this figure be multiplied by the blood volume in decilitres the total daily intake of hæmoglobin is obtained. The actual rise or fall of hæmoglobin in grams per 100 cm.³ in the corresponding period was also observed. If the drop be added and the rise subtracted from the regeneration figure the destruction of hæmoglobin in grams per 100 cm.³ blood is obtained. Summation curves were then constructed for the hæmoglobin regeneration. The shape of the curves is characteristic and indicates that the " master reaction " determining the blood regeneration and hæmoglobin intake under these experimental conditions is an autocatalyzed reversible reaction, and is therefore exclusively determined by the process of the multiplication of nucleated cells in the bone-marrow. In the course of blood disorders, periods of increasing and decreasing bone-marrow activity alternate with periods of equilibrium. The word equilibrium is used here to indicate a state of bone-marrow activity resulting in the output of a constant mass of erythrocytes each day without necessarily any relation to the rate of peripheral erythrocyte destruction, and may therefore be associated with a constant erythrocyte level, with a fall, or with a rise. The only characteristic of such a state is the constant reticulocyte concentration: the points for $\sum \frac{Hb \times R}{100} \times k$ fall therefore on a straight line.

From these data it was found that the time of repair is not identical with the duration of life of erythrocytes. It does not represent the regeneration time, but is the result of considerably increased regeneration and less considerably increased destruction. A decrease in the rate of destruction of erythrocytes occurs only in the later stages of repair and only for short periods of time. The majority of erythrocytes formed at one time die at the same time; some may be destroyed without regard to their age, but old age is the main factor which determines the life span of erythrocytes.

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STUDIES IN REFRACTORY ANÆMIA. I. The Technique and Interpretation of Sternal Puncture Biopsies. Classification

by L. S. P. Davidson, L. J. Davis & J. Innes, *Edinburgh Medical Journal*, **50**, 226–236, April 1943

The term " refractory anæmia " was first used by Bomford & Rhoads (1941) to embrace the heterogeneous group of anæmias which are refractory, temporarily or permanently, to treatment with any known hæmatinic. It includes anæmias of unknown origin and those resulting from exposure to hæmotoxic substances, but does not include the hæmolytic anæmias or anæmias which are secondary to other morbid processes, such as malignant neoplasms, leukæmias, metabolic disturbances and infections.

For the diagnosis and prognosis of these anæmias examination of the bone-marrow is essential. The following is the technique used by the authors:

About 1 cm.³ of marrow juice is aspirated from the medullary cavity of the sternum in the usual way, using a " Salah " type needle and a 10 cm.³ record syringe, and is immediately expelled into a watch-glass. After a few seconds the fluid is poured into a second watch-glass. Visible flecks of marrow tissue remain adherent to the first watch-glass; these are picked up with a fine pointed forceps and placed on microscope slides. With the aid of a spreader, the flecks are squashed and with a rapid sliding movement films are made. The degree of pressure exerted is regulated so that the marrow is squashed as lightly as consistent with the production of a satisfactory film. The number and size of the marrow particles varies considerably in different cases. In pernicious anæmia the flecks are large and abundant, while in certain types of aplastic anæmia no visible particles may be present, the appearance of the aspirated fluid differing from peripheral blood only by the presence of droplets of oily fluid. In this latter type of case films are made directly from the fluid. The films are dried in air as quickly as possible and stained by the May-Grünwald or Leishman methods. The erythrocyte precursors are divided into four types:

The Type I erythroblast is a large cell with a deep basophilic cytoplasm and a finely reticulated nucleus. Nucleoli may or may not be present. Included in this cell type are the pro-erythroblast as well as the primitive megaloblast of certain authors. Maturation of the cells, indicated by reduction in size, progressive hæmoglobinisation, and by condensation of nuclear chromatin, progresses through Types II and III to Type IV, the mature normoblast, which is often fully hæmoglobinised and with a dense pyknotic nucleus. The bone-marrow in the relapse stage of pernicious anæmia shows large numbers of Type I and II cells. In iron-deficiency anæmias Types III and IV preponderate. In marrow films from normal individuals, erythroblasts usually constitute about 25 % of all marrow cells.

A marrow smear displaying normoblastic erythropoiesis is one in which the vast majority of the erythrocyte precursors have compact condensed nuclear chromatin, while the recognition of megaloblastic erythropoiesis is based upon the presence of a greatly increased frequency of the primitive basophil erythroblasts (Type I cells), with numerous Type II cells also.

The following is the classification finally adopted, together with the numbers of cases studied in each group:

I. *With hypocellular, normoblastic marrow*
- (*a*) Secondary to exposure to toxic substances: 4 cases
- (*b*) Idiopathic, of unknown origin
  - (i) Progressive hypoplastic anæmia with fatal termination: 9 cases
  - (ii) Chronic hypoplastic anæmia, patients surviving two or more years: 2 cases
  - (iii) Relapsing hypoplastic anæmia: 1 case

II. *With hypercellular, megaloblastic marrow*
- (*a*) Occurring in pregnancy and puerperium: 10 cases
- (*b*) Idiopathic: 6 cases

III. *With cellular, normoblastic marrow and arrested myelocyte maturation*: 2 cases

REFERENCE
Bomford, R. R. & Rhoads, C. P. (1941) *Quart. J. Med.* **10**, 175

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STUDIES IN REFRACTORY ANÆMIA. II. Anæmias with Hypocellular, Normoblastic Marrows

by L. S. P. Davidson, L. J. Davis & J. Innes, *Edinburgh Medical Journal*, **50**, 355–377, June 1943

Of the 16 cases of refractory anæmias with hypocellular normoblastic marrows described, 4 were secondary to exposure to toxic substances: one had been injected therapeutically with arsenic compounds and a second with gold;

a third had been exposed occupationally to the fumes of benzol and a fourth to trinitrotoluene. The first two recovered after several months, while the third and fourth pairs died in six months and one month respectively. All had intensive treatment with iron, liver and repeated blood transfusions. The 12 remaining cases in this group were of unknown origin.

The average age at which symptoms of anæmia were first noted in this idiopathic group was high, namely 61 years. In all the cases the anæmia was macrocytic or normocytic in character. In only 2 cases were anisocytosis, poikilocytosis, ovality of outline, and macrocytosis sufficiently marked to suggest classical pernicious anæmia in relapse. In the remaining cases the films were less characteristic, although anisocytosis and a variable number of well-filled macrocytes were present, except in 2 cases which presented a normocytic picture. Without exception the hypoplastic process affected all the cells formed in the marrow: erythrocytes, granulocytes and platelets. A reticulocyte count persistently higher than 1% was present in only 3 of the cases, in which counts around 5% were persistently found.

One of the most pertinent questions arising from this study concerns the validity of accepting hypoplastic normoblastic sternal marrow film as pathognomonic of hypoplastic refractory anæmia. The authors consider that when several films made from separate flecks of sternal marrow show a consistent hypocellularity, the chances that the diagnosis of hypoplastic anæmia is correct are increased. The possibility that these findings are fortuitous is rendered still more improbable if they are confirmed by a second sternal puncture at a different site.

Thus the demonstration of a hypocellular, normoblastic sternal marrow picture in a severe anæmia, macrocytic or normocytic in character, with a colour index within or above the normal range, strongly suggests, in the absence of any evidence of a causative factor, that the condition is an anæmia of the type under discussion. If liver therapy has recently been administered, however, a reasonable period should be allowed to elapse for the occurrence of a possible reticulocyte response or a rise in the erythrocyte count before coming to a final decision. The reason for this is that, in pernicious and certain other megaloblastic anæmias, the earliest demonstrable response to liver therapy is the conversion of the megaloblastic marrow picture to a normoblastic one. However, this consideration is probably chiefly of theoretical interest, since in the authors' experience the sternal marrow pictures in pernicious and allied anæmias are uniformly hypercellular. The authors cannot find a satisfactory explanation of the mode of production of macrocytes in this type of anæmia, but believe that their presence in conjunction with a hypocellular normoblastic marrow is of diagnostic significance.

Of the 12 patients 9 died, the average period elapsing after the onset of symptoms being seven months. Of the remaining 3 cases, the ultimate outlook for 2 is poor. The other appears to have been keeping in reasonably good health for over two years in spite of a moderately severe anæmia.

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STUDIES IN REFRACTORY ANÆMIA. III. Refractory Anæmias with Cellular Marrow

by L. S. P. Davidson, L. J. Davis & J. Innes, *Edinburgh Medical Journal*, **50**, 431–443, July 1943

Of the 16 cases of refractory anæmia with hypercellular normoblastic marrows, 10 occurred in pregnancy and the puerperium. The authors have fully discussed these cases in a previous paper (1942). To summarize, they were cases of severe anæmia with hæmoglobin readings ranging from 17 to 52% (Haldane), occurring during pregnancy or the puerperium and with megaloblastic sternal marrow pictures. They displayed temporary refractory periods varying from two weeks to four months in spite of intensive parenteral liver therapy supplemented by the administration of iron and vitamin concentrates. In the majority of cases, repeated blood transfusions were necessary for the maintenance of life during the refractory period. Eventually complete recovery occurred in all cases.

The other 6 cases were of idiopathic origin. The average age of this group was 41 years. Four had a histamine-fast achlorhydria, while two had free hydrochloric acid in the resting gastric juice. The degree of anæmia was severe in all the patients when they first came under notice, and in every case the anæmia was macrocytic and hyperchromic in character, presenting morphological features similar to those of pernicious anæmia of equivalent grades of severity. Leucopenia was marked and platelets were scanty. Reticulocytes were never above 1%. The sternal marrow films when first seen were typical of pernicious anæmia in a stage of relapse, although much liver extract had already been given. With regard to treatment, the authors stress the necessity for energetic measures for maintenance of life by repeated blood transfusions together with the continuance of intensive liver therapy until recovery is obtained. The recovery of all cases in this group emphasizes the importance of persistence in treatment of this type of anæmia. Finally, 2 cases are described of severe granulopenia with moderate anæmia of unknown ætiology, which followed a chronic course of over two years and proved resistant to any form of medical treatment up to the time of writing. These cases showed a cellular normoblastic marrow with arrested myelocyte maturation.

REFERENCE

[1] Davidson, L. S. P., Davis, L. J. & Innes, J. (1942) *Brit. med. J.* **2**, 31

[1] [*see BMB* **457**]

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THE ANÆMIAS OF PREGNANCY: A Report on the Hæmatological Study of 48 Cases of Pregnancy with a Review of the Literature

by G. A. Elliott, *Journal of Obstetrics & Gynæcology of the British Empire*, **51**, 198–224, June 1944

This paper reports from the Department of Pathology of the British Postgraduate Medical School a detailed investigation of certain characteristics of the blood in (*a*) 28 cases of anæmia of pregnancy and (*b*) 20 control (without clinical anæmia) pregnant women. The investigation included: (i) erythrocyte counts, (ii) leucocyte counts, (iii) hæmoglobin estimations (Haldane), (iv) mean corpuscular volume (Wintrobe, 1930), (v) reticulocyte percentage, (vi) plasma bilirubin (Haslewood & King, 1937), (vii) mean erythrocyte diameter (Price-Jones, 1933), and (viii) erythrocyte fragility (Dacie & Vaughan, 1938).

Results are tabulated for both groups of patients. In the anæmic group, erythrocyte counts ranged from 8·27 to 5×10^6 per mm.^{3} and hæmoglobin percentages from 32 to 76. Mean corpuscular volumes were from 58 to 98μ^3. Plasma bilirubin was within normal limits. Mean erythrocyte diameters in 10 cases ranged from 6 to 7·4μ.

The chief feature of interest was a significant increase in median corpuscular fragility to normal saline, which was found in both groups. In the anæmic group, it was in some cases beyond normal limits, returning to normal after parturition.

The author discusses normal hæmotological standards in pregnancy and reviews the relevant literature. He suggests the following classification of anæmias of pregnancy:

i. *Physiological anæmia*

ii. *Deficiency anæmia of pregnancy*
- *a.* iron deficiency
- *b.* megalocytic
  1. pernicious anæmia of pregnancy
  2. megaloblastic anæmia of pregnancy and the puerperium (Davidson, Davis & Innes, 1942)
- *c.* mixed iron and pernicious-anæmia-factor deficiency

iii. *Unproven*
- *a.* acute hæmolytic anæmia of pregnancy
- *b.* protein deficiency anæmia of pregnancy (Bethell, 1936)
- *c.* vitamin B deficiency anæmia of pregnancy (Elsom, 1935, 1937)

iv. *Unclassified.*
types not included in groups i. and ii. but regarded as due to pregnancy

v. *Anæmia complicated by pregnancy*

These anæmias are discussed under the above headings, and the author concludes with a brief consideration of symptom-

atology, treatment, and the influence of anæmia of pregnancy on maternal and infantile health. There are 124 references to the literature.

REFERENCES

Bethell, F. H. (1936) *J. Amer. med. Ass.* **107,** 564
Dacie, J. V. & Vaughan, J. M. (1938) *J. Path. Bact.* **46,** 341
[1] Davidson, L. S. P., Davis, L. J. & Innes, J. (1942) *Brit. med. J.* **2,** 31
Elsom, K. O. (1935, 1937) *J. clin. Invest.* **14, 16,** 40, 463
Haslewood, G. A. D. & King, E. J. (1937) *Biochem. J.* **31,** 920
Price-Jones, C. (1933) *Red blood cell diameters,* London
Wintrobe, M. M. (1930) *Arch. intern. Med.* **45,** 287

[1] [*see BMB* **457**]

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MEGALOBLASTIC ANÆMIA OF PREGNANCY AND THE PUERPERIUM

by L. S. P. Davidson, L. J. Davis & J. Innes, *British Medical Journal,* **2,** 31–34, 11/7/42

Anæmia occurring in pregnancy and the puerperium in temperate climates, apart from the apparent anæmia caused by physiological dilution of the corpuscles resulting from the increased plasma volume of pregnancy (Dieckmann & Wegner, 1934), is usually of the hypochromic iron-deficiency type (Davidson, Fullerton & Campbell, 1935). Very rarely it may be of the classical Addisonian pernicious variety. More often it resembles this type but differs from it in certain features and has been named " pernicious anæmia of pregnancy."

In the present paper, Professor Davidson and his colleagues describe 16 cases of this latter variety of anæmia seen at the Department of Medicine, Edinburgh University. The anæmia was severe in all cases. Analysis of the peripheral blood findings indicated in many of the cases a dual deficiency of liver and iron, for while macrocytosis and a high colour index were frequent, these features were not so pronounced or so constant as in classical Addisonian pernicious anæmia. In some cases the colour indices were below unity. Free hydrochloric acid was also present in the gastric juice of many of the cases. A constant finding, however, was a megaloblastic picture in films made from the sternal marrow obtained by sternal puncture. Accordingly the name " megaloblastic anæmia of pregnancy and the puerperium " has been proposed for this type.

Ten of the cases were temporarily refractory to treatment with liver extract, iron and other hæmatinics, but persistence with such treatment, and maintenance of life with blood transfusions when necessary, eventually resulted in complete recovery in all except two cases in which death resulted from incidental causes.

REFERENCES

Davidson, L. S. P., Fullerton, H. W. & Campbell, R. M. (1935) *Brit. med. J.* **2,** 195
Dieckmann, W. J. & Wegner, C. R. (1934) *Arch. intern. Med.* **53,** 71 & 188

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THE ANÆMIA ASSOCIATED WITH INFECTION

by M. F. Saifi & J. M. Vaughan, *Journal of Pathology and Bacteriology,* **56,** 189–197, April 1944

The mechanism of production of anæmia in infections is obscure. The investigation reported in this paper was on the morphology of the blood cells in anæmias associated with infection. The peripheral blood was examined in (i) 10 mild infections (mostly furunculosis), (ii) 20 acute infections, and (iii) 14 chronic infections (of more than 3 months' duration). Bone-marrow and spleen were examined in 15 patients who died—11 cases of acute infection and 4 of chronic infection. Patients receiving a total dose of sulphonamides larger than 1 g. were excluded.

In the mild infections (group i) there was no evidence of anæmia. In 12 acute and 10 chronic infections there was anæmia of a normocytic or macrocytic type, with a colour index never above unity. There was commonly a raised reticulocyte count in chronic cases with severe anæmia. In 12 of the 15 bone-marrows examined there was evidence of increased activity, with leucopoiesis predominating. There was no aplasia of erythropoietic tissue, in which the predominant cells were primary erythroblasts and normoblasts. The degree and character of the hæmopoietic response appeared to be unrelated to type of infection, severity of anæmia, or age of patient.

On the scanty evidence available, the authors suggest that the anæmia associated with infection is due to interference of hæmoglobin synthesis resulting in arrest of erythropoiesis at the level of normoblasts and primary erythroblasts.

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ERYTHROBLASTIC ANÆMIA OF CHILDHOOD (COOLEY'S ANÆMIA) IN CYPRUS

by A. L. Fawdry, *Lancet,* **1,** 171–176, 5/2/44

Details of 20 cases of this anæmia (Cooley, Witwer & Lee, 1927) occurring in Cyprus are given, with a review of the literature and the differential diagnosis. All the patients were Greek Cypriots aged 6 months to 12 years except one case, which is fully reported, occurring in a man aged 20. Points worthy of special note were: the preponderance in males (15 out of 20 cases); the febrile onset; the occasional history of earth eating; the fact that in spite of gross physical changes, no adverse effect on mental development was found and many children seemed unusually intelligent; mongoloid features were constant in the children over the age of 6 years, and radial striation of the calvarium, although very distinctive when seen, was not as common as simple thickening.

The subjective severity of the disease was entirely a function of the degree of anæmia present, which was remarkably constant over a long period of time. It was always hypochromic and curiously lacked correlation with the age of the child, the date of onset of the illness, the bone-changes, or the number of nucleated erythrocytes in the peripheral blood. Leucocytosis was common in the younger children, but usually absent in the older; on the whole, the percentage of lymphocytes was increased and occasionally the monocytes also. Of the nucleated erythrocytes seen in the peripheral blood, the vast majority were normoblasts, but in most cases there was a small proportion of more primitive cells. Target cells were present in 50% of cases. No evidence of malaria as an ætiological agent was found. The bone marrow showed a constant marked normoblastic reaction. With regard to treatment, none was of any permanent value. Blood transfusions, iron and liver therapy were all given without success.

REFERENCE

Cooley, T. B., Witwer, E. R. & Lee, P. (1927) *Amer. J. Dis. Child.* **34,** 347

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RED-CELL SUSPENSION TRANSFUSIONS

by L. Watson, *Lancet,* **1,** 107–111, 23/1/43

In acute hæmorrhage and shock there is a profound fall in the circulating blood volume, which may be restored by the transfusion of whole blood. In these cases, however, it is not so much the replacement of the erythrocytes that is required, as the restoration of the blood volume. The use of crystalloid solutions such as normal saline results in a transitory beneficial effect, but a more prolonged effect is obtained with colloid solutions. Of the latter, plasma prepared from human blood has been found to be ideal under war conditions, as it can be well preserved without the use of refrigeration and in the dried form can be transported anywhere irrespective of temperature.

In the preparation of plasma the erythrocytes are normally wasted, but there are conditions in which erythrocytes are required but blood volume is normal and there is therefore no need for plasma. The author, working at the *Leeds General Infirmary,* has made use of the waste erythrocytes from a Regional Transfusion Centre in the treatment of anæmic patients. This is no new form of therapy as it was used as early as 1918 by Robertson, in 1937 by Castellanos & Riera, and in the present war by Beumer & Schwartzer (1939), MacQuaide & Mollinson (1940), Whitby (1941), Vaughan (1941), Davidson & Stewart (1941), and Williams & Davy (1941).

In the present paper the author reports the results of 46 transfusions of concentrated erythrocytes to 22 adult

patients, 13 suffering from anæmia following hæmorrhage, 5 from chronic anæmia and 4 miscellaneous cases.

Donors were relieved of 440 cm.³ of blood which was collected in bottles containing 100 cm.³ of 3% sodium citrate solution. This blood was allowed to stand for periods varying from 3 to 12 days at 4° C. The supernatant plasma was then siphoned off and the remaining mass was filtered through a gas mantle. The concentrated erythrocytes from several bottles were then pooled to produce a suspension containing about 290 cm.³ of erythrocytes, 157 cm.³ of plasma, and 75 cm.³ of citrate solution per bottle and furnishing about 18 grams of hæmoglobin per 100 cm.³ of suspension. This suspension could be given to the patient by the ordinary gravity drip method, but if it was more concentrated it was found necessary to provide some form of mechanical pump to maintain a constant flow. The author used only cells from Group O blood, but stresses the importance of performing a thorough cross-matching of donor's and recipient's bloods before transfusion is commenced. Transfusions were administered with the standard *Medical Research Council* equipment.

In general, the results of the treatment appeared clinically to be as good as if whole blood had been used. No systematic observations were made on the survival of transfused erythrocytes, but it was noted in one case of aplastic anæmia that cells which had been stored for 6 to 10 days survived in the circulation for 40 days, while cells stored for 12 or 13 days survived only 14 days.

If the pre-transfusion hæmoglobin level, the body weight of the recipient, and the desired rise of hæmoglobin are known, the transfusion volume can be accurately prescribed, as in the following example: Initial Hb 5 grams per 100 cm.³ Body weight 55 kg. Desired Hb level after transfusion, 15 g./100 cm.³. Hb concentration of erythrocyte suspension, 20 g./100 cm.³. Then blood volume is approximately $55/11 = 5$ litres. Total Hb in body before transfusion is $5000 \times 5/100 = 250$ grams. Total Hb in body after transfusion is $5000 \times 15/100 = 750$ grams. Therefore Hb to be added is 500 grams, and this is contained in 2500 cm.³ of the erythrocyte suspension. This method of calculating dosage is especially valuable where children are concerned.

The author recommends that (i) concentrated erythrocyte suspensions should be used when the object of treatment is to restore the oxygen-carrying capacity of the blood, (ii) erythrocytes should not have been stored for long, (iii) Group O blood should be used for making the suspension, (iv) suspensions should not be warmed before administration, (v) for suspensions containing more than 18 g. Hb per 100 cm.³ a Riddell's (1939) pump should be incorporated in the transfusion apparatus, (vi) the transfusion volume should be calculated in terms of Hb, (vii) cases of refractory anæmia should have repeated erythrocyte suspension transfusions, (viii) not more than 100 cm.³ of a suspension should be transfused to an out-patient on any one occasion.

References

Beumer, H. & Schwartzer, K. (1939) *Klin. Wschr.* **18,** 1604
Castellanos, A. & Riera, R. (1937) *Bol. Soc. cubana Pediat.* **9,** 234
Davidson, S. & Stewart, C. P. (1941) *Brit. med. J.* **1,** 644
MacQuaide, D. H. G. & Mollison, P. L. (1940) *Brit. med. J.* **2,** 555
Riddell, V. H. (1939) *Blood transfusion,* London
Robertson, O. H. (1918) *Brit. med. J.* **1,** 691
Vaughan, J. M. (1941) *Lancet,* **1,** 178
Whitby, L. E. H. (1941) *Proc. roy. Soc. Med.* **34,** 257
Williams, G. E. O. & Davie, T. B. (1941) *Brit. med. J.* **2,** 641

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THE TRANSFUSION OF CELL CONCENTRATES

by A. L. Goodall, *Glasgow Medical Journal,* **141,** 161–171, June 1944

The author reports 100 transfusions of concentrated (average 9×10^6 per mm.³) erythrocytes performed in the Glasgow and West of Scotland Blood Transfusion Service at Glasgow Royal Infirmary. The cases were of a surgical (loss of blood) and medical (blood disorders) nature and included most types for which transfusion of blood is indicated. After 1 accident in a patient with much fatty infiltration of the myocardium, administration by the drip method was used invariably, at a rate of about 60 drops per minute. There was no difficulty in infusing the concentrate by this method. There was a low incidence of reaction.

The changes resulting from the transfusions, expressed as mean rises per cells obtained from 500 cm.³ blood, were: hæmoglobin, 1·49 g. per 100 cm.³; erythrocytes, 480,000 per mm.³; packed cell volume, 3·49%.

The author regards erythrotransfusion as a safe and useful measure which is in some cases preferable to whole blood transfusion.

Other Blood Changes

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THROMBOCYTOPENIC PURPURA

by H. Evans & K. M. A. Perry, *Lancet,* **2,** 410–413, 2/10/43

This review deals with 75 cases of thrombocytopenic purpura treated at the *London* Hospital between 1927 and 1938. Thirty of the cases (40%) occurred before the age of 15 years and these were equally divided between the sexes. In ten of them, there was spontaneous recovery and, of the remaining twenty, splenectomy was successful in 5 males and unsuccessful in 4 females. One patient died with septic meningitis following chronic mastoiditis and 5 died as a direct result of the disease during the period of observation. The remaining 5 cases could not be traced. The analysis shows that this is a serious disease with a high mortality (16%), but that 10 cases out of the 30 (5 of each sex) recovered spontaneously.

Of the 45 cases (60%) which occurred after puberty, 38 were females, only one of whom made a spontaneous recovery. Splenectomy was successful in only 7 out of 13 females, the mortality during the period of observation being as high as 40%. It is evident that the disease is much more severe in women than in children, for in women the mortality is high (16 deaths in 38 cases) and the likelihood of spontaneous recovery small (1 case out of 38). Half of the deaths were due to subdural hæmorrhage.

The disease in males after puberty seems to be less severe although it is unwise to judge from the 7 patients in this series, only one of whom died—again from subdural hæmorrhage. One patient died following thyroidectomy after he had been cured of thrombocytopenic purpura by splenectomy. One case remained "unchanged" and one could not be traced. There were two spontaneous recoveries and two patients were cured by splenectomy.

In the whole series splenectomy was successful in 7 out of 7 males and in 7 out of 17 females. There were three operative deaths. Splenectomy is most successful in males before puberty and least successful in adult females, but it may at any age be a life-saving measure and is sometimes advisable in the hope of preventing subdural hæmorrhage.

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THE SOURCES OF BLOOD PLATELETS AND THEIR ADHESIVENESS IN EXPERIMENTAL THROMBOCYTOSIS

by H. P. Wright, *Journal of Pathology and Bacteriology,* 56, 151–159, April 1944

A rise in the number of circulating thrombocytes in rabbits and guinea-pigs after intravenous injection of pyridine was described by Perroncito (1920), who considered that there was an increased production and not merely a release from storage. Binet & Kaplan (1927) observed an increase in thrombocytes after injection of adrenalin in intact but not in splenectomised dogs, and suggested that the increase was due to release from a contracting spleen. Wright (1942) found that the thrombocytosis in man after surgical operations was associated with increased adhesiveness of the thrombocytes.

The work reported in the present paper was undertaken to determine whether experimental thrombocytosis was accompanied by increased adhesiveness. Methods of counting thrombocytes and measuring their adhesiveness have been described in earlier papers (Wright, 1941, 1942). The experimental procedures used in the present investigation are described and results are tabulated. In the intact rabbit, thrombocytosis was provoked both by pyridine and adrenalin, but only after pyridine was there an increased adhesiveness. In splenectomised rabbits, adrenalin had no effect, while

pyridine evoked a response which was minimal during the period when the increase in numbers and adhesiveness of the thrombocytes induced by the surgical trauma was maximal. As the thrombocyte count returned to normal, the effect of pyridine increased.

The authors conclude from these and other considerations that adrenalin produces a release of mature thrombocytes from the spleen, while pyridine increases the production of new thrombocytes from bone-marrow. It is suggested that the increased adhesiveness after pyridine and after surgical trauma is due to a reduction in the average age of the circulating thrombocytes and not to a mere increase in their number.

References

Binet, L. & Kaplan, M. (1927) *C.R. Soc. Biol., Paris*, **97**, 1659
Perroncito, A. (1920) *Hæmatologica*, **1**, 265
Wright, H. P. (1941) *J. Path. Bact.* **53**, 255
Wright, H. P. (1942) *J. Path. Bact.* **54**, 461

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HÆMORRHAGIC DISEASE OF THE NEWBORN

by E. B. S. Scobbie, *Archives of Disease in Childhood*, **17**, 175–186, December 1942

In this paper from the Department of Pædiatrics, Glasgow University, the criteria necessary for the diagnosis of hæmorrhagic disease of the newborn are discussed with reference to the literature. The author accepts Clifford's (1935) definition, "spontaneous internal or external hæmorrhage in the newborn infant in the absence of trauma, infection or other definite disease."

The records of 167 cases of hæmorrhage in the newborn infant were studied, 146 of which fall strictly within the bounds of the above definition. Eighteen of these cases were examined personally by the author. Analysis of the incidence rate corresponds in most respects with previous reports in the literature. The cases have been grouped into 3 classes according to the percentage of hæmoglobin:

Severe	Hb under 80 %
Moderate . . .	Hb 80 %–100 %
Mild	Hb over 100 %

Observations were made on the level of prothrombin in the blood in 15 cases of hæmorrhagic disease. The method used was Reid's (1941) modification of Quick's method and the level of prothrombin is expressed as the prothrombin index (Illingworth, 1939), which is

$$\frac{\text{normal prothrombin time}}{\text{patient's prothrombin time}} \times 100.$$

The following results were obtained:

In 7 severe cases	prothrombin indices of		70 %, 43 %, 35 %, 25 %, 20 %, 14 %, 5 %
In 3 moderate cases	„	„	73 %, 40 %, 18 %
In 5 mild cases	„	„	65 %, 60 %, 58 %, 45 %, 31 %

A marked variation of the prothrombin index was noted and there was a lack of correlation between the hypoprothrombinæmia and the severity of the case.

The prothrombin indices of 75 healthy newborn infants were estimated as a control. On the first day of life 24 %, on the second day 12 %, and on the third day 8 % had prothrombin indices under 50 %, as compared with 67 % of the cases of hæmorrhagic disease. However, the prothrombin index in hæmorrhagic disease is not constantly lower than in physiological hypoprothrombinæmia.

The effect of subcutaneous blood and vitamin K injections on the prothrombin index in physiological hypoprothrombinæmia and in hæmorrhagic disease was determined. Subcutaneous blood had practically no effect, whereas vitamin K produced a constant elevation although there was sometimes a delay in its action. Blood transfusion in 4 cases of hæmorrhagic disease caused a considerable increase of prothrombin.

The author attempts to analyse the effect of treatment on the mortality rate in this series. The relatively small number of cases treated with vitamin K makes this difficult. Results seem to show that intravenous blood is superior to vitamin K or subcutaneous blood as a therapeutic agent. Vitamin K therapy seems to cause a lower mortality rate than does treatment with subcutaneous blood.

The view that vitamin K deficiency explains the ætiology of hæmorrhagic disease is not supported by these findings, although it appears to be an important factor.

References

Clifford, S. M. (1935) *J. Pediat.* **14**, 333
Illingworth, C. F. W. (1939) *Lancet*, **1**, 1031
Reid, J. (1941) *Brit. med. J.* **1**, 579

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ELLIPTOCYTOSIS IN MAN ASSOCIATED WITH HEREDITARY HÆMORRHAGIC TELANGIECTASIA

by J. B. Penfold & J. M. Lipscomb, *Quarterly Journal of Medicine*, **12**, 157–167, July 1943

These authors review the literature on elliptocytosis and on hereditary hæmorrhagic telangiectasia and record the findings in a Jewish family of 32 members in 4 generations, in 5 of whom there was evidence of telangiectasia and elliptocytosis, while in 4 there was evidence of telangiectasia alone.

Those members showing elliptical erythrocytes had in addition signs of hæmolytic icterus, a delayed direct van den Bergh reaction, without an increase in fragility. Three members showed a slight degree of secondary anæmia, while 1 had an increase in erythrocytes. All 5 had over 90 % of oval erythrocytes in their blood, and although the percentage varied from person to person it seemed to be quite constant for the individual concerned. The wet film gave consistently higher percentages than the dry film and the change in the wet count on keeping was not significant (cf. sickle-cells). The authors believe that the count done on the dry blood film is not reliable, as the number of oval cells varied with the film, particularly with its thickness. The oval cells were most numerous where the film was thickest, becoming fewer as the film became thinner.

Blood from three of the subjects was diluted 200 times in hypertonic (1·2 %) and hypotonic (0·6 %) saline, and daily counts of the total and oval erythrocytes were made. In the hypertonic saline, the absolute number of oval erythrocytes diminished to a minimum at about the third or fourth day, rising again later (about the fifth or sixth day) to a figure not as high as the original. There was a diminution in the total count, gradual at first and more rapid later. Corresponding to the absolute diminution of the oval cells at about the third or fourth day, there was at that time an absolute increase in the cells which appeared circular. Similar results were obtained in the hypotonic saline solution. These observations showed that the oval shape could be changed by alteration in the physical characters of the medium.

Findings from the following hæmatological studies were normal: erythrocyte fragility test, total leucocyte count, differential leucocyte count, platelet count, bleeding time, coagulation time and Wassermann and Kahn reactions.

It is suggested that the appearance of elliptocytosis in association with hereditary hæmorrhagic telangiectasia could be satisfactorily explained if both were regarded as atavistic mutations. This view has been put forward by others in connexion with elliptocytosis and rests chiefly on the fact that most vertebrates lower than the mammals have oval erythrocytes. This is evidently the primitive type of erythrocyte.

It is not impossible that telangiectasia is also a reference back to an amphibian state in which the mucous membranes of mouth and throat regions and the skin are accessory breathing surfaces and therefore highly vascular.

The authors draw particular attention to the icteric phenomena present in their cases of elliptocytosis and point out that this feature, although seldom mentioned in text books, was present in not less than 12 % of the recorded cases. The nature of the mechanism causing the increased hæmolysis is not known.

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TROPICAL EOSINOPHILIA

by R. J. Weingarten, *Lancet*, **1**, 103–105, 23/1/43

Under this title, the author describes 81 cases of a new syndrome characterised by severe spasmodic bronchitis, leucocytosis and very high eosinophilia. The condition is

common in India and particularly affects people living near the sea. It has no apparent relation to tuberculosis or any other disease, and begins with lassitude, fever rising as a rule to 100–101° F. [about 37·9–38·3° C.] in the evening, anorexia, and usually appreciable loss of weight within a short time. After about a week of these symptoms, a dry, ineffective cough develops, with exacerbation at night. The patient's sleep is interrupted by paroxysms of coughing, and these are often accompanied by wheezing sounds audible at a distance. Gradually he develops expiratory dyspnœa which even persists during the intervals between attacks of coughing, and this stage lasts many weeks. In some cases, however, severe attacks of typical bronchial asthma occur regularly every night, usually between 1 and 5 a.m.; but the restlessness, sense of suffocation and anxiety are not so alarming as in genuine asthma. In the day-time the patient is comparatively free from coughing and breathlessness. After some weeks the temperature becomes subfebrile, and there is no further loss of weight. General weakness decreases to some extent, but more or less violent paroxysms of coughing, and in many cases asthmatic attacks, persist at night, and if no treatment is given become chronic. Physical signs, during the more typical attacks, resemble those of bronchial asthma. If there is any expectoration, the sputum is scanty, tenacious and glassy. On microscopic examination, Charcot-Leyden crystals or Curschmann spirals are rarely seen, but clumps of eosinophils are often found. During the febrile period the spleen is moderately enlarged, extending 3–5 cm. below the costal margin; it is hard, smooth and not tender. The *x*-ray picture of the lungs during the febrile period shows a distinctive disseminate mottling of both lungs, the average single focus being about the size of a split pea with moderate intensity of its central shadow and ill-defined, blurred outlines. These foci are more numerous and usually slightly larger in and near the hilar regions, and commoner in the bases than in the apices; they are not confluent but both lungs are equally affected. Adrenalin gave prompt relief in the spasmodic attacks and a course of injections of arsenicals was found to be a specific remedy.

[Carter, H. F., Wedd, G. & d'Abrera, V. St. E. (1944, *Ind. med. Gaz.* **79**, 163) have recently reported from Ceylon similar cases which were associated with the presence of parasites, including species of *Tyroglyphus*, *Carpoglyphus*, *Glyciphagus*, *Cheyletus* and *Tarsonemus*, in the sputum. They suggest that "tropical eosinophilia" may be due to infestation by these parasites.]

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TROPICAL EOSINOPHILIA

by B. G. Parsons-Smith, *Lancet*, **1**, 433–434, 1/4/44

The author reports a typical case of tropical eosinophilia, as recently described by Weingarten (1943), occurring in an English airman in Egypt. After the first two injections of neoarsphenamine, there was a recrudescence of the condition, a common occurrence in Weingarten's cases, but subsequent injections produced a dramatic, rapid and permanent cure.

REFERENCE

[1] Weingarten, R. J. (1943) *Lancet*, **1**, 103

[1] [*see BMB* **466**]

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AGRANULOCYTOSIS AND APLASTIC ANÆMIA AFTER ARSPHENAMINES

by J. W. Ferguson, *Lancet*, **1**, 334–337, 11/3/44

Since the introduction of organic arsenicals in the treatment of syphilis, there have been a number of reports of toxic effects on the hæmopoietic system, although in view of the widespread use of these compounds this would appear to be a relatively rare complication.

The author gives case records and details of blood and bone-marrow findings in 6 soldiers and seamen who developed blood dyscrasias after neoarsphenamine treatment. In the first 5 cases there was agranulocytosis, and the sixth showed depression of all marrow elements. Three of the cases were fatal, and in the 3 which recovered an increased absolute number of monocytes was found. The first 5 cases had received an average of 5 injections of neoarsphenamine, the average total dose being 2·1 g. The sixth patient had received 5·7 g. of neoarsphenamine and 2·0 g. of bismuth.

The more favourable prognosis in cases in which there is an increase of monocytes in the peripheral blood has previously been remarked (Beck, 1933; Rosenthal & Abel, 1936; Reznikoff, 1938).

The author is of the view that pentnucleotide assisted recovery in the 3 non-fatal cases.

REFERENCES

Beck, R. C. (1933) *Arch. intern. Med.* **52**, 239
Reznikoff, P. (1938) *Amer. J. med. Sci.* **195**, 629
Rosenthal, N. & Abel, H. A. (1936) *Amer. J. clin. Path.* **6**, 205

Hæmoglobinometry

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ESTIMATION OF HÆMOGLOBIN BY THE ALKALINE HÆMATIN METHOD

by J. W. Clegg & E. J. King, *British Medical Journal*, **2**, 329–333, 19/9/42

The authors of this paper, an Area Clinical Pathologist in the British Emergency Medical Service (Ministry of Health) and a Reader in Pathological Chemistry (University of London), discuss hæmoglobinometric methods in current use and give particulars of a new technique employing alkaline hæmatin as a standard.

The hæm-pigments circulating in the blood may be divided into an active fraction (reduced hæmoglobin and oxyhæmoglobin) and an inactive fraction (chiefly carboxyhæmoglobin and methæmoglobin). A satisfactory method of estimating hæmoglobin should measure both fractions. The Haldane and acid hæmatin methods may not measure total pigment with the degree of accuracy desirable. Commercial standards are often unreliable and may fade. The pyridine hæmochromagen method is rejected by the authors because of the objectionable nature of the reagents employed.

Criteria of a satisfactory method are described. These include the measurement of total pigment, reasonable accuracy, simplicity, the use of non-toxic reagents, and some permanent and easily reproducible standard. The alkaline hæmatin method of Wu eliminates errors which arise in the acid hæmatin procedure due to lipids, the colloidal nature of acid hæmatin, and the presence of inactive hæmoglobin. Alkaline hæmatin has the advantage of being a true solution and is easily prepared both from hæmoglobin and from crystalline hæmin. Hæmin is easy to prepare in a pure state from hæmoglobin, and its iron content may be estimated with accuracy. Thus it appeared to satisfy the authors' criteria for a suitable standard, and was investigated for this purpose.

Sixteen samples of crystalline hæmin were prepared from ox and human bloods and the iron content of each was determined. Solutions of alkaline hæmatin were prepared from each sample. The specific extinction coefficients were found to agree closely. The intensity of colour was proportional to the concentration of hæmin iron, and impurities failed to influence the colour.

Several forms of hæmoglobin occur in normal blood, some of which are resistant to alkali denaturation. Whatever the proportion of alkali-resistant hæmoglobin in a blood sample, if a 1 in 100 dilution in decinormal soda is prepared and placed in a boiling-water bath for five minutes, all the hæmoglobin is converted into alkaline hæmatin. Similar treatment converts all inactive forms of hæmoglobin into alkaline hæmatin. The colour developed is not significantly influenced by the amount of lipoid or plasma in the sample.

Determination of hæmoglobin in blood samples, based on the assumption that 1 mg. hæmin iron in the standard was equivalent to 1 mg. hæmoglobin iron in the test, yielded results uniformly about 30% higher than determinations based on the oxygen-carrying power of the same sample. It was then found that solutions prepared from hæmin and blood gave colours whose absorption curves differed slightly in the red portion of the spectrum.

Samples of human and ox hæmoglobin were prepared and their iron content was determined. Solutions of alkaline hæmatin were prepared from each sample and their specific extinction coefficients and absorption curves were determined. The curves derived from the hæmoglobin solutions were identical with that from blood, and the colour produced was

30 % higher than would be anticipated from the iron content, when compared with hæmin standards.

Thus hæmin is not a theoretical standard in the sense that four molecules of hæmin should give the same colour as one molecule of hæmoglobin; but it can be employed satisfactorily as an artificial standard. The colours of the solutions are almost identical in the concentrations used for the test, and are matched with ease and accuracy. The standard appears to be unchanged during several months.

Details of methods for the estimation of total hæmoglobin, using hæmin as a standard, are given, together with instructions for the preparation of standard solutions equivalent to 100 % of the Haldane, Sahli, and Haden scales.

The authors had employed this method for many months and found it to give excellent results. Where many determinations were made daily it was quicker and more reliable than other methods they had used.

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A SIMPLE INEXPENSIVE PHOTOELECTRIC HÆMOGLOBINOMETER

by G. H. Bell & E. Guthmann, *Journal of Scientific Instruments*, **20**, 145–146, September 1943

This paper, from the Institute of Physiology, University of Glasgow, describes a simple and inexpensive colorimeter which is unaffected by variations in the light source or photocell characteristics. To avoid calculating the extinction-coefficient (E) at each estimation, a pointer attached to the lamp moves along a scale which is calibrated directly in E values obtained by a simple calculation. With the lamp at the maximum distance D_0 from the photocell and with water alone in the glass cell, the galvanometer deflexion is noted; blood is added to the water in the glass cell and the lamp is pushed up to a distance D_1 from the photocell where the photocell current is the same as before. The $E = \log D_0^2/D_1^2$. Details of the construction and calibration as a hæmoglobinometer are given. All the component parts are readily available, and inexpensive. A modification of the simple instrument is also described using a balanced circuit with two photocells in parallel opposition to cancel out the light fluctuation when the lamp is supplied from either direct or alternating current mains.

Plasma and its Constituents

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PLASMA PROTEIN VALUES IN INFANTS

by E. M. Hickmans, E. Finch & E. Tonks, *Archives of Disease in Childhood*, **18**, 96–97, June 1943

This report from the *Children's* Hospital, Birmingham, deals with the results of plasma protein determinations in 20 premature babies and 180 normal full-term infants and young children up to 40 weeks old attending infant welfare centres. Estimations were made by a micro-Kjeldahl method, followed by Nesslerization, on specimens of 0·01 ml. of plasma obtained from heparinized blood from a heel prick. [Whether the amount of heparin used was sufficient to affect the nitrogen figures is not stated.]

It was found that in the first 2 weeks of life the infant has between 4 and 7 g. per 100 ml. plasma protein, although " in the majority of instances " the value was below 6 g. per 100 ml. Values for premature infants in the first four weeks of life were between 3·7 and 5·4 g. per 100 ml. The range for full-term infants over 2–3 weeks old was 4·7–7·4 g. per 100 ml. In infants over 10 weeks old " most of the values lie in the upper half of this range, viz. between 6 and 7·4 g. per 100 ml."

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THE EFFECT OF THE PLASMA PROTEINS UPON THE SEDIMENTATION RATE OF HUMAN BLOOD

by C. M. Gordon & J. R. Wardley, *Biochemical Journal*, 37, 393–397, September 1943

It has been known for some time that the sedimentation rate of erythrocytes is determined in part by the protein constituents of the plasma. No clear correlation has, however, been found between the sedimentation rate and the concentrations of the various plasma proteins although Frazer & Rennie (1941) found a fall in plasma albumin and, to a smaller extent, increase of fibrinogen, to be associated with an increased sedimentation rate. In this paper from the Pathological Department of the *Lake* Hospital, Ashton-under-Lyne, the problem has been approached by building up ' pathological plasmas ' with protein fractions prepared from normal plasma, and observing the behaviour of normal cells suspended in them. In most cases the protein concentrations of the solutions were about 3 g./100 ml. of 0·85 % NaCl and the cell volume suspension was 20 %. Sedimentation was observed in Westergren tubes, usually at the end of 1 hour.

The most rapid sedimentation rates were found with fibrinogen and euglobulin, the order being fibrinogen (100 mm. in 1 hour) > euglobulin > globulin > albumin (1·5 mm. in 1 hour). The results were, however, affected by the treatment to which the protein fractions were submitted during their isolation. The " slower " proteins when mixed with " fast " proteins did not merely reduce the sedimentation rate by acting as a diluent. Nucleoprotein, albumin and the " globoglycoid " fraction of albumin, retarded the sedimentation rate of cells suspended in solutions of fibrinogen. Sedimentation was slightly accelerated by total globulin and its euglobulin fraction. Sedimentation can take place in the absence of fibrinogen which, however, plays the chief part in determining the rate.

From two experiments it was concluded that an artificial mixture of proteins from " normal " plasma can closely resemble naturally-occurring pathological plasma in its effect upon sedimentation. By altering only the globulin fractions in " artificial tuberculous plasma " the sedimentation rate could be changed from 94 to 26 mm. When crystalbumin was substituted for the total albumin of " artificial nephritic plasma " the sedimentation rate increased from 15·0 to 145 mm.; when globoglycoid was substituted it was reduced from 15·0 to 1·0 mm.

REFERENCE

Frazer, T. N. & Rennie, J. B. (1941) *Brit. J. exp. Path.* **22**, 81

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CHANGES OCCURRING IN PLASMA AND SERUM ON STORAGE AND THEIR PHYSIOLOGICAL EFFECTS

by H. P. Gilding & M. E. Nutt, *Journal of Physiology*, **102**, 446–470, April 1944

These experiments reported from the Department of Physiology in the University of Birmingham deal with the toxic effects in cats following the rapid injection of plasma or serum, either human or from cats, which has been stored for over 14 days. From 0·5–2 ml. of plasma or serum, which had been stored for periods up to 18 months, was rapidly injected warm, usually into the internal saphenous vein of cats anæsthetised with " dial." Injection at the rate of 0·6 ml./kg./minute was followed by similar but much less striking effects. No toxic manifestations followed injection of fresh (prepared within 45 minutes of bleeding) plasma.

Although the cat was found to be the most suitable animal in which to demonstrate the toxic properties of stored serum and plasma, toxic reactions were observed in dogs, in rabbits and in two apparently healthy men. In the animals, records were taken of systemic and pulmonary arterial pressure, venous and intrapleural pressure, intestinal and limb volume, and total and differential leucocyte count.

The following reflex effects were noted both in anæsthetised and in decerebrate cats within 20–30 seconds of giving the injection—vagal inhibition of the heart with actual arrest in some cases, profound fall in the systemic and an increase in the pulmonary arterial pressure, altered respiration which sometimes ceased, increased peristalsis, micturition, defæcation, vomiting, pupillary constriction, opisthotonos and a leucopenia which was followed after some hours by leucocytosis. Necropsy revealed that the capillaries of the lungs and suprarenals and, to a minor extent, of the liver were packed with polymorphonuclear leucocytes, a finding which has been described previously in anaphylaxis, but it was established that this phenomenon was not responsible for the reflex response to plasma injections. Injection of plasma into the splenic vein was not followed by the massive reflex effects but still produced a leucopenia suggesting that the liver can protect the animal from the toxic effects of stored

plasma, most of which are abolished by vagotomy or by atropine. Cross-circulation experiments supported the finding that the effects mentioned above were reflexly produced. It was established that the vagus, although the most important, cannot be the only sensory nerve involved in the reflex, which may be initiated by pressure changes in the pulmonary circulation.

All these phenomena, which resemble those occurring in anaphylactic shock, were shown to be the result of storing plasma or serum for some 3–4 weeks. The clotting process seems to play no part in the development of toxicity and the breakdown of platelets is not responsible. The activity is associated with an albumin which is precipitated by 66–75 % saturation with ammonium sulphate (and by other methods) and represents not more than 8 % of the total protein of the original serum or plasma. The different conditions described following the injection of stored human or cat plasma into cats " may merely represent different methods of eliciting a general physiological response to the injection of foreign matter into the circulation."

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A STUDY OF THE ACCURACY OF SERUM PROTEIN ESTIMATIONS AND OF DIURNAL VARIATIONS IN THEIR LEVEL

by M. Dyson & G. Plaut, *British Medical Journal*, **2**, 6–7, 3/7/43

In this investigation from the North-West London Blood Supply Depot, serum protein was determined by the micro-Kjeldahl method of Pregl (1937) and by the method of King and his associates (1937, 1942) which employs Kjeldahl digestion followed by Nesslerization and colorimetric determination. In order to test and compare the two methods, ten estimations on each of ten samples of the same serum were made, the procedure being repeated with each of ten different sera with protein values between 6·03 and 7·40 g. per ml. giving 100 estimations in all by each method. The results obtained by both methods were analysed separately in each of two ways: (*a*) by computing the grouped differences and (*b*) by determining the average standard deviations. Both methods of treatment showed that the micro-Kjeldahl technique is the more accurate. With the latter method, changes of less than 0·15 g. per 100 ml. should be ignored, but changes of 0·15 to 0·20 are probably, and those over 0·20 are almost certainly significant, whereas with King's method a difference must exceed 0·40 g. per 100 ml. before it can be considered significant.

To determine whether there is a significant diurnal variation in serum protein under " ordinary working conditions " 5 samples were taken at two-hourly intervals between 9 a.m. and 6 p.m. from 20 healthy persons of ages between 18 and 50. No great variations were found by either method. This finding does not agree with the published results of other authors, but Dyson and Plaut emphasise " that many variations in serum protein recorded in the literature are not significant and that changes in the character of tissue and circulating fluid cannot be calculated from changes in serum protein unless accurate methods with a known technical error are employed and standard conditions of posture and exercise adopted."

REFERENCES

Pregl, F. (1937) *Quantitative organic microanalysis*, London

King, E. J., Haslewood, G. A. D. & Delory, G. E. (1937) *Lancet*, **1**, 886

King, E. J., Haslewood, G. A. D., Delory, G. E. & Beall, D. (1942) *Lancet*, **1**, 207

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FIBRINOGEN DEFICIENCY AS A FACTOR IN HÆMORRHAGIC DISEASE

by E. C. Allibone & H. S. Baar, *Archives of Disease in Childhood*, **18**, 146–153, September 1943

This paper, from the *Children's* Hospital and the Department of Pædiatrics at Birmingham University, contains the description and discussion of two fatal cases of fibrinogen deficiency in infants. A review of the literature showed that some fifteen cases had previously been reported, ten of which occurred in infants or children. In five of these cases, and in one now reported, a low thrombocyte count was associated with a low plasma fibrinogen. The authors accept the view that fibrinogen deficiency can exist in two forms. Congenital fibrinopenia is characterized by a tendency to bleed from birth, although in cases which survive with a continuously low or absent plasma fibrinogen, the bleeding becomes periodic for reasons which are at present not understood.

The authors' first case belonged to this group, the ætiology of which is quite unknown. The plasma fibrinogen was 0·11 g. per 100 ml. and the coagulation time exceeded 4 hours. The second case was regarded as belonging to the second group—acquired transient afibrinogenia following congenital obliteration of the bile ducts, complete obstructive jaundice, and biliary cirrhosis. The fibrinogen was initially nil but reached 0·34 g. per 100 ml. 3 days before death. It was considered that evidence exists for the production of fibrinogen by the bone-marrow as well as by the liver, which may explain the thrombocytopenia found in some cases, the gradual increase in plasma fibrinogen in the second case with extensive liver damage, and the fact that gross liver damage can exist with normal plasma fibrinogen. The relationship between prothrombin, fibrinogen, and other coagulation factors is discussed.

Circulatory Dynamics

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SYNCOPE IN BLOOD DONORS

by F. C. Poles & M. Boycott, *Lancet*, **2**, 531–535, 7/11/42

In this paper from the British Army Blood Transfusion Service the authors have analysed the causes of fainting in blood donors. Of their first 10,000 donors only 2·9% fainted, but of these 14% collapsed after their return to work, an accident which might have serious consequences. All donors were kept lying down for 15 minutes after bleeding and were given a cup of tea and a biscuit, and they then remained sitting for another 5 minutes before returning to their work. The common clinical findings of those who fainted were pallor, sweating, slow breathing and a low blood pressure. The systolic pressure may still be below 100 mm.Hg. an hour after bleeding and may take 2 hours to return to normal. Fainting affected both sexes equally but was much commoner in young people. Greenbury (1942), however, found that young female donors were most likely to faint than males especially in the age-group 18–25 years.

The rate of withdrawing blood and the heat and humidity of the surroundings did not influence the incidence of fainting, nor was it affected by moderate anæmia or low blood volume. On the other hand, there was a definite connection between the amount of blood withdrawn and subsequent syncope, and fainting was much more common in donors who had given more than 440 cm.³ of blood. All the donors who fainted had a low blood pressure after bleeding and in some the blood pressure fell much more rapidly after 440 cm.³ had been removed. In the normal donor, the systolic pressure hardly fell at all and had started to rise 15 minutes after the operation, but nearly all those who suffered from delayed syncope still had a falling systolic pressure after 15 minutes. The donors who were subject to delayed fainting attacks also showed an alteration in blood pressure when they sat up after the operation, the systolic pressure falling while the diastolic rose slightly at first and then followed the systolic pressure. This phenomenon differs from true orthostatic hypotension in which there is a simultaneous fall in both systolic and diastolic pressures. In general the delayed fainting attacks resembled vaso-vagal attacks which have been described by Sir Thomas Lewis (1932).

The measures advocated by the authors for the prevention of syncope in blood donors are as follows:

i. Avoidance of bleeding when the donor is fatigued.

ii. Provision of food and fluid before as well as after taking the blood if the donor's last meal has been taken more than 4 hours previously,

iii. Not taking more than 440 cm.³ of blood (not more than 350 to 400 cm.³ if the donor weighs less than 7 stone [44 kg.]).

iv. Administration of 1 litre of normal saline, before bleeding, to donors who are likely to be dehydrated, e.g., those coming from hot workshops. This was found to effect a marked reduction in the number of these workers who fainted.

v. Taking a series of blood pressure readings on all donors who have fainted on a previous occasion. If the systolic pressure falls after bleeding and continues to fall after sitting up, the donor should be given further rest even if he feels well.

REFERENCES

Greenbury, C. L. (1942) *Brit. med. J.* **1**, 253
Lewis, T. (1932) *Brit. med. J.* **1**, 875

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VENOUS PRESSURE DURING VENESECTION AND BLOOD TRANSFUSION

by J. F. Loutit, M. D. Mollison, & E. D. van der Walt, *British Medical Journal*, **2**, 658–661, 5/12/42

In man few observations have been made on the changes in venous pressure during the withdrawal and administration of blood, although Altschule & Gilligan (1938) observed that transfusion of saline at rates above 20 cm.^{3} a minute caused a rise in venous pressure proportional to the amount of fluid given. Immediately after the end of such transfusions the venous pressure fell rapidly, reaching the original level within 20 minutes. Murphy (1941), also using saline, noted a rise in venous pressure during the first half hour of transfusion followed by a fall which he attributed to vasodilatation.

In the present investigation, from a London Blood Supply Depot, venous pressure changes (recorded by inserting a 16 S.W.G. intravenous needle into an antecubital vein and connecting the needle with a manometer) were observed in healthy individuals during venesection, in unselected hospital patients during transfusion, and in a group of patients with pulmonary disease, before, during, and after transfusion.

Venesection was performed on 20 healthy blood donors, 430 cm.^{3} of blood being removed from each over an average period of 4 minutes. In every case there was a fall in venous pressure during bleeding ranging from 13 to 80 mm. of water with an average of 43 mm. This fall usually commenced after 100 to 200 cm.^{3} of blood had been withdrawn, continuing until the end of the venesection. After completion of bleeding the venous pressure rose again slowly, but in no case did it reach the pre-bleeding level by the end of 30 minutes. The changes in blood-pressure and pulse-rate were variable.

The venous pressure was estimated during the transfusion (administered by gravity with the *Medical Research Council* apparatus) of 30 unselected hospital patients who were given 500–1000 cm.^{3} of citrated blood at rates varying from 7–45 cm.^{3} a minute (average 18·5 cm.^{3} per minute). In 19 out of 30 cases the venous pressure rose by more than 20 mm. of water during the transfusion, but only in 6 cases did the rise exceed 50 mm. of water. There was apparently no constant relationship between the rate of transfusion and the rise of venous pressure, although proportionately more cases showed a rise at the faster rates. In the cases in which the venous pressure rose, the increase occurred progressively during the transfusion and was approximately proportional to the amount of blood transfused. Contrary to the findings of Altschule & Gilligan, however, it was observed (in 10 cases) for 10 to 30 minutes after transfusion that, while the venous pressure fell gradually, it did not return to the pre-transfusion level during the period of observation. No constant changes in blood-pressure or in pulse-rate were observed in 26 cases before and after transfusion. Only 3 of the 30 cases showed obvious signs of peripheral vasodilatation during transfusion.

In 13 patients suffering from various chronic chest diseases, who were transfused with 800 to 1000 cm.^{3} of blood at an average rate of 23 cm.^{3} a minute, both the venous pressure and the vital capacity were measured at the beginning and end of transfusion. Six of the 13 cases showed a rise in venous pressure during transfusion and all but one a reduction in vital capacity at the end of transfusion (average reduction 230 cm.^{3}). The respiratory rate was increased in 3 of the patients by more than 4 respirations per minute but only 2 patients complained of symptoms.

The authors discuss the mechanism of production of the rise in venous pressure and the reduction in vital capacity which occurred during these transfusions, and they conclude that they are not due to cardiac embarrassment but merely indicate that the larger veins and the lung capillaries are accommodating the extra fluid.

REFERENCES

Altschule, M. D. & Gilligan, D. R. (1938) *J. clin. Invest.* **17**, 401
Murphy, F. D. (1941) *J. Urol.* **45**, 654

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FAINTING IN BLOOD DONORS

[Unsigned] *British Medical Journal*, **1**, 279–283, 26/2/44

This is the text of a report to the *Medical Research Council* prepared by a Subcommittee of the Blood Transfusion Research Committee. The large-scale bleeding of donors that has been undertaken since the outbreak of war gave rise to an interest in the nature and causation of fainting during or after the donation of blood. Such incidents are a source of delay and inconvenience, and an investigation was instituted with the objects (i) of identifying factors associated with fainting in blood donors and (ii) of determining the frequency of delayed faints. A record card was designed and provided to bleeding centres in England and Scotland. Blood transfusion officers were asked to complete a card for (*a*) every donor who fainted during a bleeding procedure, (*b*) the next donor bled by the same officer (these constituted an unselected group who did not faint).

The data received were submitted to a statistical analysis, and are summarized in 11 tables in this report. Figures were obtained for a total of 697 donors of whom 362 fainted and 335 did not. The incidence of fainting appeared to be lower in men than in single women, and slightly lower in single than in married women. There was no evidence that age, length of waiting period at the centre before bleeding, or operational difficulties affected the incidence of fainting. Menstruation was present at the relevant time in an equal number of fainters and non-fainters. Fainting was less common in the fortnight preceding menstruation than in the week after its cessation.

There was little difference between various occupational groups, and lack of food could have been a relevant factor only in a small proportion of the total number of faints. A high proportion of fainters gave a history of fainting either at a previous donation or on some other occasion. Among those who fainted, 13 % lost consciousness, nearly 8 % vomited, and 79 % had only mild symptoms.

Returns from 4,212 donors who were asked to describe their feelings after bleeding showed that only 14 % experienced some discomfort. Only 1·2 % of men and 1·8 % of women recorded delayed faints.

The conclusions reached are that: (i) there was no remediable factor except the exclusion of those with a known tendency to faint, (ii) the incidence of delayed faints was very small.

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BLOOD VOLUME IN CHRONIC ANÆMIA BY A CONCENTRATED CORPUSCLE-HÆMOGLOBIN METHOD

by J. McMichael, E. P. Sharpey-Schafer, P. L. Mollison & J. M. Vaughan, *Lancet*, **1**, 637–640, 22/5/43

This paper is by members of the Committee on Wound Shock and Blood Transfusion of the *Medical Research Council* and is based on work done at the *British Postgraduate Medical School* and at two of the four Blood Supply Depots which serve the London area.

It has long been known that in chronic anæmia the blood volume is below normal. In acute anæmia due to hæmorrhage the return of the blood volume to normal may also be prolonged. Methods of measuring the blood volume have not been widely used and are all open to certain criticisms on theoretical grounds. This comparison of three methods which can safely be used on seriously ill patients is therefore welcome.

The "concentrated corpuscle-hæmoglobin" method (CC–Hb) described by Hill in 1941 and the "concentrated corpuscle-differential agglutination" method (CC–DA) of Ashby (1925) both involve the administration of a blood

transfusion as part of the technique and are therefore particularly suitable for use with anæmic patients.

The former (CC–Hb) involves the transfusion of a known volume of blood, from which most of the plasma has been removed and which is therefore especially rich in erythrocytes. The hæmoglobin content of this blood, and also of the patient before and after the transfusion, is measured. A simple formula gives the patient's original blood volume, on the assumption that no gross displacements of plasma, either into or out of the circulation, occur during the transfusion.

The formula is

$$x = \frac{V(Hb_v - Hb_2)}{Hb_2 - Hb_1}$$

where x = patient's original blood volume
V = volume of blood transfused
Hb_v = hæmoglobin percentage of injected blood
Hb_1 = patient's Hb percentage before transfusion
Hb_2 = " " after "

Ashby's original CC–DA method has been modified by Mollison and others, and is said to be capable of giving results of the same order of accuracy as an ordinary erythrocyte count. It is only suitable for patients of blood groups A, B or AB. A known volume of blood from a group O donor is transfused. After the transfusion a sample of the patient's blood is taken and treated with an appropriate blood-grouping serum. This agglutinates the patient's cells but does not affect the group O cells of the donor. The agglutinated masses are removed by coarse filtration and the remaining cells are counted in the ordinary way. The blood volume at the end of the transfusion is given by the formula:

$$\text{Final blood volume (cm.}^3\text{)} = \frac{\begin{pmatrix}\text{Volume of blood}\\ \text{transfused}\end{pmatrix} \times \begin{pmatrix}\text{erythrocyte count}\\ \text{of transfused blood}\end{pmatrix}}{\text{Count of donor cells in patient's blood}}$$

Certain precautions needed in using this method are given in the papers already quoted.

The Evans-blue dye method of Gibson & Evans (1937) as modified by Harington, Pochin & Squire (1940) consists in giving an intravenous injection of 5 cm.³ of a 0·24 % solution of Evans-blue dye and then taking three samples of venous blood at half-hourly intervals. The concentration of dye in the serum is estimated colorimetrically and the plasma volume calculated by extrapolation backwards of the three points on the disappearance curve of the dye.

The results obtained by these methods are of great interest. Twenty-two cases were examined by the CC–Hb method, 12 of whom were suffering from various forms of chronic anæmia and 10 from acute anæmia due to hæmorrhage. In all cases low blood volumes were found.

Five of these chronic cases and one of the acute cases were also examined by the CC–DA method and a very close correlation between the two estimates was obtained. In no case did the difference between the two methods exceed half a litre. In four cases the dye method was compared with the CC–Hb method and again good correlation was found.

A comparison of the low blood volumes recorded in the present series of cases of chronic anæmia and those published by Gibson indicate that the lowering is proportional to the severity of the anæmia.

Another interesting observation made in this series of patients was that the diastolic blood pressures were rather low but the systolic pressures were normal. This compensatory mechanism might be due to a marked reduction of the peripheral capacity of the circulatory bed. If this is the body's response to a low blood volume it might be desirable to produce the same effect by drugs in cases of surgical shock.

REFERENCES

Ashby, W. (1925) *Ann. intern. Med.* **35**, 516
Gibson, J. G. & Evans, W. A. (1937) *J. clin. Invest.* **16**, 301
Harington, C. R., Pochin, E. E. & Squire, J. R. (1940) *Clin. Sci.* **4**, 311
Hill, D. K. (1941) *Lancet*, **1**, 177
Mollison, P. L. & Young, I. M. (1940) *Quart. J. exp. Physiol.* **30**, 313

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THE DETERMINATION OF THE BLOOD VOLUME IN MAN WITH EVANS-BLUE (" T-1824 ")

by L. J. Davis, *Edinburgh Medical Journal*, **49**, 465–483, August 1942

In the first part of this paper from the Department of Medicine, Edinburgh University, the various methods for the determination of the blood volume in man and the special advantages of the dye method are briefly considered. The author then discusses the various factors influencing the results obtained with the dye method, including choice of dye, mixing time and disappearance-rate of the dye, methods of estimation, hæmatocrit, and gives an excellent review of the relevant literature. He concludes that a spurious degree of accuracy is often attributed to existing methods of blood-volume determination, and that the evaluation of a blood-volume technique should be based upon its freedom from avoidable sources of error, its simplicity in operation, and the constancy with which comparatively accurate values can be obtained, rather than upon claims to a high degree of absolute accuracy involving tedious and time-consuming manipulations which may themselves introduce further errors.

In the second part of the paper the author describes the details of a simplified blood-volume technique, the results of which are presented. The technical procedure is essentially as follows: A sample of control blood is withdrawn from the patient and 5 cm.³ of a 240 mg. per 100 cm.³ solution of the blue azo dye " T-1824 " are then injected through the same needle. Exactly 10 minutes later a test sample of blood is withdrawn from the opposite arm. Of this blood, some is mixed with anti-coagulant mixture for hæmatocrit determination and the remainder is allowed to clot. The concentration of the dye in the test-serum is subsequently estimated by means of the " spekker " photo-electric absorptiometer, serum from the control blood being used as a " blank." Details are given of a simple and reliable method of preparing a control dye-serum solution of known concentration for the purpose of checking the readings.

The results obtained with 11 normal male subjects are tabulated, the average total blood volume being 5,071 cm.³, the volume per kilogram of body weight being 76·7 cm.³, and per metre² of body surface 2,897 cm.³ These results and their standard deviations are compared with those of 4 previous authors. In 5 of the patients the determinations were repeated and the differences between the pairs of readings were seen to be small (in no case more than 2·7 %).

The results of blood-volume determination in 10 patients suffering from pathological conditions are also given. These included 3 cases of severe anæmia and 5 cases of polycythæmia vera. In some of these cases the blood-volume determinations were repeated after appropriate treatment had been instituted. The findings accorded with those of previous investigators.

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THE DETERMINATION OF PLASMA VOLUME BY THE EVANS-BLUE METHOD: The Analysis of Hæmolysed Plasma

by C. J. O. R. Morris, *Journal of Physiology*, **102**, 441–445, April 1944

The procedure developed in the *London* Hospital for the determination of plasma volume by the Evans-blue method has previously been described (Crooke & Morris, 1942). Even very slight hæmolysis in the samples of plasma may cause an error of about 5 % in the result since, during the extraction process, hæmoglobin is converted into a pigment (probably acid hæmatin) whose light absorption is in the same spectral region as that of Evans-blue. In conditions in which information about the plasma volume may be required it is not always possible to obtain specimens of plasma entirely free from hæmolysis, and a method was therefore sought in which this source of error could be eliminated. By an ingenious procedure, for details of which the original paper should be consulted, an optical method of correction for this error has been devised. The practical application of this method to plasma-volume determinations is described.

REFERENCE

Crooke, A. C. & Morris, C. J. O. R. (1942) *J. Physiol.* **101**, 217

BOOKS, MEMORANDA, REPORTS

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OPERATIVE DENTAL SURGERY

by J. B. Parfitt & W. E. Herbert. Fifth edition. London, Edward Arnold & Co., 1944. 408 pages ; 203 illustrations. £1 10s. [£1.5]

This book first appeared in 1921, being the substance of a course of lectures delivered at *Guy's* Hospital Dental School. Since that time successive editions have reflected the advances made in operative dentistry. Perhaps the most important recent development in prosthetic dentistry is the introduction of acrylic resins, both for dentures and restorative work, and the authors include a chapter dealing with its use in crowns, bridges and other restorations. The remainder of the book has been carefully revised and brought up to date ; new illustrations and additional references have been included.

Chapter headings: (i) operative dentistry ; (ii) infection and cleanliness ; (iii) control of saliva ; (iv) conservative treatment of parodontal disease ; (v) ionic medication ; (vi) the onset and growth of dental caries ; (vii) treatment of dental caries ; (viii) the teeth of children ; (ix) conditions of permanence of tooth fillings, and general principles of cavity preparation ; (x) pain-saving in the filling of teeth ; (xi) the filling materials ; (xii) filling teeth with gutta-percha ; (xiii) dental cements and their uses ; (xiv) the translucent cements ; (xv) amalgam ; (xvi) cohesive gold ; (xvii) non-cohesive gold ; (xviii) gold inlays ; (xix) bridges ; (xx) fused porcelain restorations ; (xxi) pulpless teeth and their treatment ; (xxii) the operation of crowning ; (xxiii) acrylic resin restorations ; (xxiv) the operation of extraction.

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BROMPTON HOSPITAL REPORTS

Volume 12

London. Published by the Research Department of the Hospital, 1943. 163 pages. 8s. [£0.4]

This volume contains a collection of papers recently published from the Hospital. Dr. Margaret Macpherson [1] contributes an article on childhood infection and its relation to adolescent and adult pulmonary tuberculosis, in which she sums up the extensive work carried out by the research department of the *Brompton* Hospital during the past 14 years. A. Tudor Edwards [2] gives an authoritative survey of the modern treatment of traumatic hæmothorax. There are two illustrated articles by C. Price Thomas and W. P. Cleland [3] on the operation of thoracoplasty for pulmonary tuberculosis, which give a full account of the technique and results.

R. C. Brock [4] describes the production of aseptic obliterative pleurisy by injecting silver nitrate into the pleural cavity. The same author, in another paper, discusses the treatment of tuberculous empyema and supplements his discussion with a most instructive analysis of the results of various therapeutic measures.

Other interesting papers in this volume include one by R. C. Wingfield on the control of tuberculous infection, and an original contribution by A. F. Foster-Carter [5] on the anatomy of the bronchi.

[1] [*see BMB* 282] [2] [*see BMB* 45] [3] [*see BMB* 266 & 267]
[4] [*see BMB* 44] [5] [*see also BMB* 245 & 246]

482/68

INDUSTRIAL MEDICINE

edited by Sir Humphry Rolleston & Alan A. Moncrieff. London, Eyre & Spottiswoode (Publishers) Ltd., 1944. 202 pages. 16s. [£0.8]

Eleven of the 18 contributions comprising this symposium on industrial medicine have been specially written for it ; the remainder were first published in the *Practitioner*, December 1942, and have been revised before republication.

The war has brought about a huge increase in the number of persons engaged in industry, and this has led inevitably to an increase in the incidence of disease directly attributable to industrial causes. By the foresight of the Minister of Labour, provision was made in 1940 for the employment of whole-time medical officers and nurses in factories large enough to warrant them ; part-time medical officers are employed in smaller establishments. This closer association of the practitioner with the factory is certain to continue after the war, and such contact with " occupational medicine " on the part of the general practitioner will inevitably benefit both the employer and the employee.

This symposium will serve as an excellent introduction for the general practitioner desiring to acquaint himself with the commoner industrial diseases and with problems of general hygiene and administration. It contains the following contributions: (i) introduction: industrial medicine and the general practitioner (Sir David Munro) ; (ii) industrial poisons (H. M. Vernon) ; (iii) industrial dermatoses ; diagnosis and treatment (A. D. K. Peters) ; (iv) chest disease in industry (A. J. Amor) ; (v) miners' nystagmus (W. J. W. Ferguson) ; (vi) toxic anæmia (Ethel Browning) ; (vii) the treatment of the injured workman (W. Gissane) ; (viii) back-strain (F. W. Holdsworth) ; (ix) neuroses in industry (E. H. Capel) ; (x) malingering (D. C. Norris) ; (xi) nutritional problems related to industrial workers (H. A. Krebs) ; (xii) adolescents in industry (Sir Henry Bashford) ; (xiii) fatigue and boredom (May Smith) ; (xiv) lighting problems (E. W. Murray) ; (xv) ventilation and heating (T. Bedford) ; (xvi) welfare services (R. R. Hyde) ; (xvii) the works ambulance room (P. Pringle) ; (xviii) factory law in relation to health and welfare (A. I. G. McLaughlin).

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VARICOSE VEINS, HÆMORRHOIDS, AND OTHER CONDITIONS

Their Treatment by Injection

by R. Rowden Foote. London, H. K. Lewis & Co. Ltd., 1944. 119 pages; 54 illustrations. 12s. 6d. [£0.625]

At the time of its introduction, the injection treatment of varicose veins was welcomed as a useful new method of alleviating this condition, but sufficient data have now been published to show that a high recurrence rate must be expected. This finding has led many to abandon injection therapy in favour of conservative treatment or high ligation. The author of this monograph, who is physician in charge of the Injection Clinic, Royal Waterloo Hospital, considers that too many cases are at present treated operatively, and that many require treatment by injection only. He discusses both aspects of this question and gives a full account of the ætiology of varicose veins and of their treatment by injection and operation. In addition, the injection treatment of hæmorrhoids, hydrocele, hernia and other conditions is described. This book, which is well illustrated, provides a good picture of injection therapy at the present time.

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RECENT ADVANCES IN ANÆSTHESIA AND ANALGESIA

(Including Oxygen Therapy)

by C. Langton Hewer. 5th edition. London, J. & A. Churchill, 1944. 343 pages ; 141 illustrations. 18s. [£0.9]

A new volume or new edition in the *Recent Advances* series, to which this book is an outstanding contribution, is almost always an event of some importance. Although the fourth edition appeared in 1943, sufficient new work has been reported to make possible this new edition. Besides an account of modern anæsthesia and analgesia, it gives new information on anæsthesia for thymectomy, general analgesia with ethyl chloride and trichlorethylene, intravenous general analgesia with procaine, improved apparatus for controlled respiration, pethidine analgesia in obstetrics, pressure infiltrators, fractional caudal block, suction from oxygen cylinders, etc. There is an additional chapter on anæsthetic charts and records. Each chapter includes a useful list of references for further reading ; there is room for improvement in the method of presentation of these references.

Chapter headings: (i) theoretical aspects of inhalational anæsthesia ; (ii) premedication ; (iii) nitrous oxide and the hydrocarbon gases ; (iv) carbon dioxide and helium ; (v) modern apparatus for the administration of the " gas " anæsthetics ; (vi) recent work on the ethers ; (vii) the halogen-containing anæsthetics ; (viii) recent developments in endotracheal anæsthesia ; (ix) the explosion risk in anæsthesia ; (x) intravenous anæsthesia and analgesia ; (xi) general aspects of local analgesia ; (xii) drugs used in local analgesia ; (xiii) recent advances in the technique of local analgesia ; (xiv) the present position of spinal analgesia ; (xv) collapse and resuscitation ; (xvi) anæsthesia and analgesia for neuro-surgery ; (xvii) anæsthesia and analgesia for dental surgery ; (xviii) anæsthesia and analgesia for endoscopy, for nasal and oral surgery, for war wounds involving the mouth, and for operations upon the pharynx and larynx; use of suction ; (xix) anæsthesia and analgesia for thyroid and thymic surgery ; (xx) anæsthesia and analgesia for thoracic surgery ; (xxi) anæsthesia and analgesia for abdominal surgery ; (xxii) anæsthesia and analgesia in obstetrics—resuscitation of the new-born ; (xxiii) anæsthetic sequelæ ; (xxiv) psychological aspects of anæsthesia and analgesia ; (xxv) oxygen therapy ; (xxvi) anæsthetic charts and records.

482/71

ECONOMY IN THE USE OF DRUGS IN WAR-TIME

Medical Research Council War Memorandum No. 3. Revised second edition. With an Appendix on Economy in the Use of Bactericides. London, H.M. Stationery Office, 1944. 16 pages. 3d. [£0.0125]

The first edition of this Memorandum, issued in March 1941, expressed the considered opinions of the Therapeutic Requirements

Committee, which was appointed in September 1939 by the *Medical Research Council* in consultation with the Ministry of Health. The object of the publication was to give to those concerned with the supply of drugs an indication of the relative therapeutic importance of the drugs in ordinary use, and to members of the medical profession some information regarding the availability in war-time of the materials which they were accustomed to prescribe.

In preparing the first edition, the scope was extended, after consultation with the Ministry of Agriculture and Fisheries, to include veterinary applications of drugs.

In November 1941 the First Supplement to this Memorandum was issued, in order to bring the recommendations up to date. In April 1942 a Memorandum on Economy in the Use of Bactericides, prepared by the Therapeutic Requirements Committee, was issued by the *Medical Research Council* to the medical and pharmaceutical press.

Since the publication of the first edition of the present Memorandum, effect has been given to its recommendations, by means of Addenda to the *British Pharmacopœia* 1932, Supplements to the *British Pharmaceutical Codex*, and the *National War Formulary*. A new edition is now considered advisable, in consequence of changes in the supply position of many drugs, the introduction of new remedies, and increased knowledge of drugs as the result of war-time experience.

482/72

REGIONAL ANALGESIA

by H. W. L. Molesworth. London, H. K. Lewis & Co. Ltd., 1944. 90 pages ; 42 illustrations. 8s. 6d. [£0.425]

The author of this book has had much experience in the use of regional analgesia, and he discusses fully the advantages and disadvantages, the indications and contra-indications for this form of anæsthesia. While not to be regarded as a textbook on the subject, this little volume contains much practical information and many useful illustrations.

Chapter headings : (i) general principles ; (ii) methods of regional analgesia ; (iii) the upper extremity ; (iv) the lower extremity ; (v) the head and neck ; (vi) the thorax ; (vii) the abdomen ; (viii) spinal analgesia.

482/73

REPORT OF INTER-DEPARTMENTAL COMMITTEE ON MEDICAL SCHOOLS

published by the Ministry of Health and the Department of Health for Scotland. London, H.M. Stationery Office, 1944. 312 pages. 4s. 6d. [£0.225]

To meet the requirements of the proposed National Health Service, fundamental changes will be necessary in the methods of recruitment and training of future doctors. This was foreseen by the appointment of the Inter-departmental Committee on Medical Schools, under the chairmanship of Sir William Goodenough. The Ministry of Health and the Department of Health for Scotland have now published the findings of this committee (the " Goodenough Report ").

The principal recommendations of the committee are: (i) drastic overhaul of undergraduate training: more attention to be paid to social medicine, the promotion of health, and the prevention —as well as cure—of disease, to children's health and to mental health ; (ii) co-education in all medical schools, and sex equality in hospital appointments ; (iii) greatly increased Exchequer grants for medical education and research, and more financial help for students ; (iv) reform of examination system ; (v) compulsory hospital appointments after qualification and before entry into independent practice ; (vi) changes in the policies and organization of medical schools and teaching hospitals ; more whole-time teachers, and salaries for part-time teachers ; (vii) a comprehensive system for the training of specialists ; (viii) development in London of a world centre for post-graduate medical education and research ; (ix) linking of all major hospitals with teaching centres.

The proposed changes will take some time to become fully effective. An increased number of teachers and additional accommodation and equipment will be necessary. The training of students can be conducted only under the ægis of a university and in institutions that conform to university standards. For this reason the committee suggests the abolition of 4 medical schools which it feels cannot adequately meet the needs of modern medical education. The unit of organization for undergraduate medical education should be a medical teaching centre consisting of a university medical school with a group of teaching hospitals in as close proximity as possible to it, and such clinics of the health service of the district as should be used for teaching purposes. While the governing body of each constituent of a teaching centre should retain full authority in its own field of responsibility, the policy, administration and activities of each institution should be so interrelated with the other constituents as to provide a single unit in the field of medical education and research.

More whole-time appointments to the teaching staff and the payment of salaries to clinical teachers are among changes proposed.

At present there is great inequality in the distribution of medical schools, with a maximum concentration in central London. It is suggested that *Charing Cross*, *St. George's*, *Middlesex*, and the *Royal Free* hospitals (and their respective medical schools) should all consider moving to sites further from the centre of London. As far as the establishment of new medical schools is concerned, the committee concludes that " the financial resources and the suitably qualified teachers likely to be available will be wholly needed for a long time for the development of existing schools to their full capacity."

Before the establishment of a new school " there must first be proof of a national need for another school. . . . The aim should be to secure better facilities for the existing schools and to improve and develop the exceptional facilities of London for post-graduate medical education."

The kind of post-graduate training and experience required by intending specialists and the means whereby it can be obtained have been examined by the committee as questions of great importance, both in the operation of the health service and in the sphere of medical education. On the assumption that the qualifications and standards of specialists are determined by some central machinery and that the primary requisite will be approved post-graduate training and experience extending over not less than 4–5 years after registration, the committee makes various suggestions, including the following: (i) that, while holding hospital appointments, the intending specialist should be regarded as a trainee and be given adequate time for reading, reflection and research ; he must not be over-burdened with routine work and he must be adequately remunerated ; (ii) usually, before he begins to specialize, he should have at least six months' resident clinical experience of general medicine or surgery ; (iii) every trainee should have the opportunity to devote himself for a period to the extension of his scientific knowledge by laboratory work and study, and provision should be made, by such means as travelling fellowships for trainees, to obtain the benefit of working for a time in suitable hospitals and medical schools abroad. Post-graduate study should be a regular and recognized feature of general practice.

A scheme is outlined for the development of the exceptional resources of London for the post-graduate education of medical practitioners from all parts of the world. This scheme involves the reconstitution of the *British Postgraduate Medical School* on a federal basis embracing a comprehensive range of post-graduate institutes in all branches of medicine.

Young research workers should mainly recruit themselves, and when a research worker has proved his ability and has chosen research as a career, he should be given reasonable security in this career. Public grants to medical schools and teaching hospitals must include basic research grants ; extra grants will also be needed for the support of special investigations.

One of the most important recommendations is that each student, after passing his qualifying examination, shall complete a 12 months' period of hospital appointments before being admitted to the Medical Register and allowed to enter independent practice.

In the past, insufficient attention has been paid to social medicine. " A new orientation of medical education, a big expansion of the social work of teaching hospitals and radical changes in the outlook and methods of most of the teachers " are necessary if students are to be fitted to become health advisers and members of a national health service. Improvements are also necessary in the teaching of child-welfare work, and " in many schools the experience which students gain of maternity work falls far short of what is necessary." Training in psychiatry, also, has failed to keep pace with the growing realization of the important place which the subject should occupy ; an adequate department of psychiatry is essential in each medical centre.

" Unsuitability for a medical career should be the sole barrier to admission to a medical school." Co-education has proved successful in all medical schools outside London and it should become the practice in every medical school. It is recommended that the payment to any school of a government grant should be conditional upon the school admitting a reasonable proportion of women students ; all hospital appointments of qualified practitioners should be filled by open competition and sex should not be a bar to appointment. Grants to medical students should compare more favourably than at present with grants to students in other faculties. Such grants should be adequate in amount and should extend over the whole of the period of training. Arrangements for scholarships and grants need simplification, and medical schools should have larger funds at their disposal to help students and to encourage recruits.

It is estimated that the supply of doctors necessary in civilian practice in Britain is within the capacity of existing schools provided that those schools which are below an economic size are expanded ; it is considered that a school of normal size is one able to admit 100 students a year.

The committee estimates that, including the outlay of teaching hospitals on facilities for teaching and research, the total of capital expenditure that should be incurred may, within ten years, amount to £10,000,000 at pre-war costs. In addition, the amount of recurrent grants will have to be increased yearly from £700,000 a year, the approximate amount before the war, until within ten years the amount reaches between £3,000,000 and £4,000,000 a year at pre-war values. At this level recurrent grants would represent about 2 % of the estimated cost in the first year of the National Health Service.

The report, which is unanimous, is signed by Sir William Goodenough, Sir John Stopford, Professor T. R. Elliott, Dr. A. M. H. Gray, Professor J. Hendry, Professor A. V. Hill, Sir Wilson Jameson, Professor J. R. Learmonth, Sir Ernest Pooley and Dr. Janet Vaughan.

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COMMON SKIN DISEASES

by A. C. Roxburgh. Seventh edition. London, H. K. Lewis & Co., Ltd., 1944. 454 pages ; 192 illustrations. 18s. [£0.9]

Seven editions of this book have appeared in 12 years, and this latest edition maintains the high standard, both of text and illustrations, set by its predecessors. The book gives a short account of the common dermatoses and their treatment. The most important changes made in the new edition are in the section on

industrial dermatitis, which has been expanded on account of its increasing prevalence, and in the chapter on the avitaminoses, which has been rewritten. The section on scabies takes account of the important work done on this condition since the war. Two addenda deal with (i) immersion foot, a condition which has become increasingly prevalent during the last few years and one on which much has been written; and (ii) the results of the use of penicillin in dermatology. The author has already recorded his experiences with penicillin and considers that it will prove of especial value in the treatment of certain staphylococcal and streptococcal infections of the skin and in the control of sycosis.

Chapter headings: (i) general ætiology and pathology; (ii) signs, symptoms and general diagnosis; (iii) general treatment; (iv) congenital affections of the skin; (v) skin affections due to physical causes; (vi) dermatitis due to chemical causes; (vii) drug eruptions; (viii) diseases due to animal parasites; (ix) diseases due to vegetable parasites; (x) diseases due to filterable viruses; (xi) syphilis; (xii) diseases due to obstruction of vessels; (xiii) neurodermatoses; (xiv) toxic eruptions; (xv) eczema, Besnier's prurigo, cheiropompholyx; (xvi) psoriasis and pityriasis rubra; (xvii) lichen planus; (xviii) diseases of hair and nails; (xix) diseases of sebaceous and sweat glands; (xx) tumours; (xxi) atrophy and sclerosis; (xxii) vesicular and bullous eruptions; (xxiii) the erythrodermias; (xxiv) avitaminoses.

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REPORT ON THE INCIDENCE OF RICKETS IN WAR-TIME

by the British Pædiatric Association. Ministry of Health: Reports on Public Health and Medical Subjects, No. 92. London, H.M. Stationery Office, 1944. 36 pages. 9d. [£0.0375]

At the invitation and with the co-operation of the Ministry of Health, the British Pædiatric Association undertook a combined clinical and radiological examination of 5,283 children between the ages of 3 months and 18 months in 23 centres in Britain, to ascertain whether or not there was any war-time change in the incidence of rickets. Positive radiological evidence of rickets was reported in 106; from these figures it is shown that the rate of incidence of rickets diagnosed radiologically was 2½% before 6 months of age, 4% during the first year of life, and negligible over this age.

The clinical diagnosis of the condition varied in different areas from nil to 61%; only a small proportion of clinically positive cases was confirmed radiologically. Most observers agree that severe rickets with deforming bone changes has almost disappeared from Britain, and with this disappearance the clinical conception of rickets must undergo some modification. A disturbance of calcification demonstrable radiologically but not clinically may need a new terminology. Among radiologically negative children 81½% were breast fed, whilst among radiologically positive children 69% were breast fed. These findings confirm the fact that breast feeding alone, although important in the prevention of morbidity and mortality, cannot be relied upon to prevent the occurrence of rickets. Nevertheless, rickets is more likely to appear in artificially-fed than in breast-fed babies.

It is shown that, of children free from rickets on radiological examination, 85½% had received cod-liver oil or other vitamin D preparation, whereas the figure for the radiologically positive children was only 73%.

The report concludes that comparison with previous surveys affords no evidence of any war-time increase in radiological rickets. Nevertheless, certain cases in the groups examined did show an incidence which still gives scope for better prophylactic measures, of which the Government's national "cod-liver oil" compound is an important one. The report calls attention to the difficulties associated with the early diagnosis of rickets and the present-day position of the nutritional problem of adequate calcification of bone in the active growing child.

482/76

DEPARTMENT OF HEALTH FOR SCOTLAND

Summary Report for the Year ended 30th June, 1944

Edinburgh, H.M. Stationery Office, 1944. 23 pages. 4d. [£0.016]

This report shows that, in spite of war-time conditions, the general standard of health in Scotland is being maintained. Although there is a slight rise in the infant mortality rate, the stillbirth and maternal mortality rates are the lowest yet recorded. Notifications of new case of pulmonary tuberculosis continued to increase, and are now more than 50% above the figure for 1938; this may be due in some measure to mass miniature radiography and other improved diagnostic facilities.

Among infectious diseases there was a small decrease in venereal disease, although the number of new cases continues to cause concern. There is a striking decrease in the figures for diphtheria, no doubt due to immunization of the child population. Recently there has been a tendency for the proportion of immunized children to fall, and it is proposed to launch another campaign to encourage immunization.

The health of school children is being maintained and, in some instances, improved. The Miners' Centre established in 1943 for the rehabilitation of disabled miners has already demonstrated its value. Progress is reported in the development of plans for post-war health services and for an attack on the serious problems of unsatisfactory housing.

The authorities realize that the successful operation of modern measures for improving the health of the nation depend on the co-operation of individual citizens; for this reason the Scottish Council for Health Education, constituted in 1943, must in time be of great value in promoting and encouraging health education.

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A STUDY OF VARIATIONS IN OUTPUT

by S. Wyatt (assisted by R. Marriott, W. M. Dawson, D. E. R. Hughes & F. G. L. Stock). Emergency Report No. 5 of the Industrial Health Research Board, Medical Research Council. London, H.M. Stationery Office, 1944. 16 pages. 4d. [£0.016]

Investigations were made on representative groups of about 200 workers in each of several factories engaged on war production, with a view to ascertaining the effect of a reduction in working hours upon output. The reduction was small (from about 54 to 51 hours per week) but this relaxation of working time was followed by an increase of 3% in the output per hour during the first and second four-week periods after the change, rising to 6% in the next four weeks. The general trend was for weekly output to be higher with the shorter working day. Changes in output often depended, however, on other factors than the effect of shorter hours, i.e. changes in the type or design of product, mechanical difficulties and machine breakdowns, variations in quantity and quality of the material used, working conditions, changes in type and layout of machines.

While it is shown that a shortened working week may be expected to be followed by increased output and decreased absenteeism, changes in the design of machines, of the lay-out of work and of the form or type of product are particularly important factors influencing output. "Machines should be made for men: not men forcibly adapted to machines. Such alterations of design, to produce all round favourable effects, must be so shaped that the demand on the individual worker does not diverge significantly from what may be reasonably expected from the average abilities of the working group."

482/78

THE TRAINING OF THE NURSERY NURSE

Interim Report of the National Council for Maternity and Child Welfare published by the National Society of Children's Nurseries, 117 Piccadilly, London, W. 1. 19 pages

At the request of the National Council for Maternity and Child Welfare, a group committee was formed by the National Society of Children's Nurseries to report on the training of nursery nurses. The interim report of this committee includes the following recommendations: (i) prospects and conditions of service for nursery nurse students should be set out and made public, and the co-operation of headmistresses should be secured to ensure the enlistment of suitable candidates; (ii) the minimum age of admission for nursery training should be 16; (iii) candidates should have received a secondary-school education or provision should be made for suitable elementary school-girl candidates to receive further general education; (iv) periodical medical examination and careful watch and record of the nursery student's health is essential; (v) the maximum hours of work should be 96 a fortnight with a minimum of 4 weeks' holiday per annum; (vi) adequate recreational facilities should be provided; (vii) the training course should be for 2 years, including a 3 months' probationary period; (viii) the curriculum should cover an elementary study of everything concerned with the physical and mental development of the child, together with simple ailments and first aid, and instruction in domestic subjects; (ix) in addition to the training staff working in the nursery, the services of specialist lecturers (doctors, health visitors, child psychologists, etc.) should be available; (x) nurseries wishing to give training should be subject to initial and periodic inspection by properly qualified inspectors. The report also recommends that all students should be required to sit for both practical and theoretical examinations and that there should be one national examining board to award a diploma to successful candidates; such a diploma should qualify for the post of (a) staff nurse in a nursery, (b) children's nurse in private households, and it should be considered as a basis for further training for higher posts in children's nurseries, hospital nursing, nursery school teaching, social welfare work, etc.

482/79

VITAL STATISTICS IN THE TROPICS

by P. Granville Edge. Foreword by M. Greenwood. London, Baillière, Tindall & Cox, 1944. 188 pages. 12s. 6d. [£0.625]

"Thus Dr. B. has, for our instruction, imitated on a small scale the vast experiment which is constantly going on, and destroys thousands of men, women and children all over England. . . . More than two millions of people live in London over sewers and cesspools. . . . Will the London Boards of Works stop the experiment? Are they, like Dr. B. convinced and satisfied? Will they bring their common sense to bear on this question? . . . The sweet odours that enter the country are taxed; and every one has witnessed the admirable zeal of Her Majesty's customs' officers in their search for Eau de Cologne. If a tax could be levied upon the odours of another description, bearing some proportion to the evil they do, it would be much more productive."

Statisticians excepted, few people would accept the view that statistical literature is ever light, easy and interesting reading. And even amongst statisticians of the modern schools, few would guess correctly the source of the above quotation to be the annual reports of the Registrar-General for England and Wales. Yet it will be found on pp. xix and xx of his report for 1858 and, selected at random by the reviewer, typifies the style of many of the reports in the period 1856 to 1875. The present-day civil servant, circumscribed by sub-committees appointed by committees of the Joint Conferences on this and that, would find some difficulty in obtaining sanction for publication, if he presented his report in such terms as those quoted. Nowadays, partly owing to the growth of a specialised technique, statistical reports have acquired a language

of their own, and couched in stereotyped and cautious terms, are not easy reading for the man in the street.

From the point of view of both popular and statistical appeal Major Edge is fortunate in his subject, in that he deals with peoples and places far more primitive than was our own land at the time when Registrar-General Graham was advocating the abolition of London cesspools as a means of lowering the prevailing high mortality rates. The only thing uninteresting about his book is the title. It would better have been termed "The romance of the tropics as expressed in vital statistics."

The general arrangement is readily appreciated. Under every heading—numbering of the population, systems of birth and death registration, or the collection of other statistical information—the author discusses first, methods in countries which have had long experience of such systems. Their evolution is briefly traced, and the uses to which the information demanded of the citizen is put, for his own advantage, are clearly demonstrated. From this aspect alone, the book may well be read not only by colonial medical officers of health for whom it is obviously primarily intended, but also by the general public, who will be cheered to find that the numerous forms they fill in, often have a definite advantage for mankind.

Next he demonstrates the futility of attempting to impose the same methods of census-taking and registration on indigenous populations, and the fallacies which may appear in the results of these methods are persisted in against native prejudices, It is in so doing that he takes the reader with him to the less trod corners of the earth—Borneo, Malaya, Nigeria, the Andaman Isles—to name only four—and presents the collection of vital statistics more in the guise of books on travel. Of the difficulties of obtaining records of plural births he writes:

"The Wabugwe tribe of Tanganyika also believed in the ill luck surrounding twins, and were accustomed to expose such infants to die in the bush. But curiously enough, a neighbouring tribe, the Wambulu, had no such prejudices, and as the birth rate of this tribe was low, they would freely adopt abandoned children; so the custom had developed under which Wabugwe twins would be exposed on the borders of the Wambulu territory, where they were taken by this tribe and brought up as their own."

In addition he supplies constructive suggestions as to how these many difficulties, by studying native customs, may be to some extent overcome. For example:

"In the post-natal period curious customs and practices are sufficient in number and variety to remind the observant stranger of the presence in the community of newly-born children. These evidences are seen in the food *tabus* of nursing mothers, the wearing of special abdominal bandages, and special ornaments, the smearing of bodies with earth, oil, etc. and the special signt of recent motherhood which provide exemption from tribal duties involving heavy labour."

Entertained so hugely, the reader will be in the mood merely to smile at certain inconsistences as evidencing the author's enthusiasm. Throughout each chapter he stresses the importance of proceeding slowly in the introduction of new schemes of registration, and the care needed to avoid arousing the resentment of the natives by demanding information relating to their tribal life. Yet he suggests:

"wherever possible some or all of the following facts should be recorded whenever the birth of a child is registered—race, tribe, colour, age at marriage, duration of marriage, occupation, religion, number of children (*a*) born alive and living (*b*) born alive but dead, (*c*) still-born; and whether the newly-born child was the mother's first-born, or otherwise (2nd, 3rd etc.)."

When it is remembered that although birth registration has been compulsory in England for over a century, figures relating to still-births have been available only since 1931; and that it was only in the face of a good deal of resentment by the natives of England that it was made compulsory in 1939 to supply information regarding the age of the mother and the number of previous children when registering a birth, the anomaly is striking.

A foreword by Professor Major Greenwood praises the book, and may be of use in correcting any impression some readers may form that the collection and tabulation of data and the calculation of simple birth and death rates are all that is needed in the compilation of a statistical report.

482/80

THE SURGERY OF ABDOMINAL TRAUMA

by Geoffrey E. Parker. London, J. & A. Churchill Ltd., 1944. 120 pages; 10 illustrations. 10s. 6d. [£0.525]

The reports of 94 cases of abdominal and abdomino-thoracic wounds seen and treated by the author during the Italian and Tunisian campaigns form the basis of this work. As the preface points out, problems are encountered on the battlefield which, apart from accidents on the road and in the factory, are without parallel in peace-time surgery. The book has therefore been written to assist surgeons who, although experienced in general surgery, are about to undertake military surgical work, and also for those practising surgery in industrial areas. The author does not consider that there is such a thing as the clinical diagnosis of an "absolutely hopeless abdominal injury"; such cases should always be given the chance that operation provides. He also stresses the view, widely held, that the time interval from wounding until the patient stops moving must be cut down to a minimum by taking the surgeon as near to the line as possible; the period required for rest, resuscitation, etc., before the optimum level is reached, varies between half an hour and six hours or longer. The quicker the patient is brought to the operability level, the better his chances of survival.

This is a useful little book, worthy of the attention of surgeons serving with the Armed Forces. Chapter headings: (i) diagnosis of abdominal injury; (ii) pre-operative treatment and resuscitation; (iii) general operative considerations; (iv) technique of the repair of abdominal viscera; (v) statistics; (vi) case notes; (vii) post-operative treatment and its principles.

482/81

NOTABLE NAMES IN MEDICINE AND SURGERY

by Hamilton Bailey & W. J. Bishop. London, H. K. Lewis & Co. Ltd., 1944. 202 pages; 142 illustrations. 15s. [£0.75]

For those who prefer to take their history of medicine in small doses, this is an admirable book, as valuable to nurses and other medical auxiliaries as to doctors themselves. There is something interesting on every page. Several thousand proper names exist in medical terminology, and while we are familiar with "Unna's paste" and "Koplik's spots," with "Ménière's disease" and "Trendelenburg's position," few of us know much about the men who have been thus eponymized. A well-known surgeon has collaborated with the sub-librarian of the Royal Society of Medicine in the production of the book, which consists of brief sketches of the lives and work of 83 men and women who have made notable contributions to medicine and whose names are perpetuated in medical terminology. A portrait is included with each sketch and the book is interspersed with many other interesting illustrations. The book will serve as an excellent stimulant to a further interest in the study of the history of medicine.

482/82

MODERN TREATMENT YEAR BOOK 1944

A Year Book of Diagnosis and Treatment for the General Practitioner edited by C. P. G. Wakeley. London, Medical Press & Circular, 1944. 300 pages; 17 plates, 15 illustrations. 15s. [£0.75]

This volume, the tenth in the series, comprises 42 articles, contributed by well-known authorities in Britain. The first 21 articles deal with a variety of subjects in medicine, surgery, gynæcology and obstetrics, while the remaining contributions discuss modern methods of treatment of diseases and injuries associated with war. The aim of this volume is to provide a concise account of recent progress in treatment and it is especially suitable for the doctor in the Armed Forces and the busy general practitioner.

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